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MOLECULAR CORRELATES OF COCAINE ADDICTION AND METHODS FOR THEIR USE

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made in part with U.S. Government support under National Institute on Drug Abuse (DA13772 and DA15941). The U.S. Government may have certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates generally to molecular correlates of drug addiction and more specifically to the identification of molecular targets for use in diagnosing and treating addictive disorders.

BACKGROUND OF THE INVENTION

In 1998, the National Household survey on Drug Abuse estimated that 1.8 million American were current cocaine users, 595,000 frequent users, and 2.4 million occasional users. With no significant decreases since 1992, these figures represent a persistent problem. Understanding the neural mechanisms that mediate cocaine abuse is critical for improving existing therapies and developing new treatment therapies. Chronic cocaine use results in particular intense euphoria and persistent drug dependence. In humans, the propensity to use cocaine is influenced by both positive (euphoric, pleasurable effects) and negative (withdrawal, depressed mood states, and drug cravings) consequences, including the development of neuroadaptive changes in specific brain regions (Gawin (1991) *Science* 251:1580-1586).

The characteristics of the addictive disorder although specific to cocaine itself, may generalize to other drug dependencies. Current understanding on the neuroadaptive processes stemming from chronic cocaine administration is based largely on rodent models of human drug taking, such as intravenous self-administration. However, reliance on such models has failed to yield useful pharmacotherapies for human cocaine addiction. Although the behavioral utility of

rodent models for studying addictive processes is acknowledged, the biological utility of such models is questionable based on the anatomical and molecular complexity of the primate brain.

Accordingly, there remains a need for the identification of molecular targets in the diagnosis and treatment of cocaine addiction.

SUMMARY OF THE INVENTION

The present invention provides methods for identifying genes and their expression products as targets in the diagnosis and treatment of addictive disorders. The targets are identified based on their differential expression in subjects affected by an addictive disorder in comparison with control subjects. Genes whose expression level is significantly increased or significantly decreased in subjects affected by an addictive disorder in comparison with control subjects are identified, along with their expression products, as screening targets for use in methods of identifying drugs for treating addictive disorders.

The invention provides an improved method for screening for therapeutic compounds for use in treating addictive disorders, where the improvement comprises the use of a target identified by the method described above.

In another aspect, the present invention provides methods for producing an expression profile having values representing the expression levels of genes whose expression is correlated with addictive disorders. The method comprises determining the expression level of one or more genes in a sample from subjects affected by an addictive disorder, determining the expression levels of the same genes in control subjects, and identifying genes whose expression level is significantly increased or significantly decreased in subjects affected by the addictive disorder in comparison with control subjects.

The present invention also provides methods for predicting whether a compound will be addictive in humans. The methods comprise the steps of providing a reference expression profile associated with an addictive disorder, where the expression profile comprises values representing the expression level of one or more genes whose expression is associated with the addictive disorder in subjects affected by the addictive disorder, providing a test expression profile comprising values

representing the expression level of said one or more genes in a sample comprising cells that been contacted with the compound; and determining whether said test expression profile shares sufficient similarity to said reference profile. When the test expression profile is sufficiently similar to the reference profile, it is predicted that the compound will be addictive in humans.

The invention identifies genes that are differentially expressed in cocaine overdose victims in comparison with control subjects, and provides a method of screening for therapeutic compounds for use in treating addictive disorders using these genes. The method comprises screening for modulators of genes that are differentially expressed in cocaine overdose victims in comparison with control subjects. Reports have shown that common molecular pathways, including the stimulation of dopamine transmission in the limbic system, underlie the addictive nature of a number of drugs including opiates, ethanol, nicotine, amphetamine, and cocaine. *See*, for example, Koob and Nester (1997) *Journal of Neuropsychiatry and Clinical Neurosciences* 9:482-497; Wise and Bozarth (1987) *Psychological Review* 94:469-492; Di Chiara and Imperato (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85:5274-5278; Nestler (1997) *Current Opinion in Neurobiology* 7:713-719; and Self and Nestler (1995) *Annual Review of Neuroscience* 18:463-495; each of which are herein incorporated by reference. Accordingly, genes having differential expression in cocaine overdose victims are targets for the diagnosis and treatment of a number of addictive disorders.

In another aspect, the invention provides methods for evaluating a candidate drug to determine whether it will have therapeutic efficacy in treating an addictive disorder. The method comprises the steps of determining the expression level of one or more genes in a first sample from a subject affected by an addictive disorder prior to treatment with the candidate drug, wherein expression of said one or more genes is associated with the addictive disorder; determining the expression level of said one or more genes in a second sample from the subject following treatment with the candidate drug, and comparing the expression level measured before and after the treatment to determine the change in the expression level of said genes following treatment with the candidate drug. If the drug results in a change in the expression levels of the genes associated with the addictive disorder, it results in a prediction that the candidate drug will have therapeutic efficacy in treating the disorder.

Compositions for use in the methods of the invention are also provided. Compositions of the invention include an array comprising a substrate having a plurality of addresses, where each address has disposed thereon a capture probe that can specifically bind a nucleic acid molecule that is differentially expressed in subjects affected by cocaine addiction and a computer-readable medium having a digitally-encoded expression profile comprising one or more values representing the expression levels of a gene that is differentially expressed in cocaine overdose victims.

Compositions of the invention also include a kit for predicting whether a compound will be addictive in humans and a kit for evaluating the therapeutic efficacy of a candidate drug. The kits comprise an array having multiple addresses, wherein each address has a capture probe that can specifically bind a nucleic acid molecule that is differentially expressed in subjects affected by an addictive disorder; and a computer-readable medium having a digitally-encoded expression profiles having values representing the expression of a nucleic acid molecules detected by the array.

The methods of the invention have been applied to cocaine addiction to identify candidate targets for improving currently available treaments. Accordingly, one aspect of the invention is the use of the identified target genes in drug screening for drugs to treat addictive disorders.

DESCRIPTION OF THE FIGURES

Figures 1A and 1B: A. Ionotropic glutamate receptor subunit protein levels in VTA and l-SN of cocaine overdose victims. Membrane fractions were isolated as described in the Experimental Procedures and 10 mg were separated on 10% SDS-PAGE to assess glutamate receptor subunit immunoreactivity. Data are expressed as mean (± S.E.M.) of the percent of control values per amount of protein loaded. Asterisks indicate a significant difference ($P<0.05$). B. Representative bands from two cocaine overdose victims (+) and two control subjects (-) for each subunit.

Figures 2A and 2B: A. CREB protein levels in the cytosolic and nuclear fragments of VTA and l-SN in cocaine overdose victims. Cytoplasmic and nuclear fractions were isolated as described in the Experimental Procedures and 15 mg were

separated on 10% SDS-PAGE to assess CREB immunoreactivity. Phospho-CREB immunoreactivity was not detectable in either the cytoplasmic or nuclear fractions. Data are expressed as mean (± S.E.M.) of the percent of control values. * indicate significant difference ($P<0.05$). B. Representative bands from two cocaine overdose victims (+) and two control subjects (-).

Figures 3A and 3B: A. Levels of G-protein subunits in VTA and l-SN of cocaine overdose victims. Cytoplasmic fractions were isolated as described in the Methods and 10 mg were separated on 10% SDS-PAGE to assess G-protein subunit immunoreactivity. Data are expressed as mean (± S.E.M.) of the percent of control values per amount of protein loaded. Asterisks indicate a significant difference ($P<0.05$). B. Representative bands from two cocaine overdose victims (+) and two control subjects (-).

Figure 4. Comparisons of ionotropic glutamate receptor immunoreactivity in brain regions following limited and binge cocaine self-adminstration and withdrawal. Post hoc comparisons reveal statistically significant differences at $p<0.05$. C, control; B, binge; W, withdrawal.

DETAILED DESCRIPTION OF THE INVENTION

The present inventions now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the invention are shown. Indeed, these inventions may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Like numbers refer to like elements throughout.

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific

terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

The present invention demonstrates changes in gene expression levels in neuronal populations in subjects affected by an addictive disorder in comparison with control subjects. Changes in gene expression levels were detected in human post-mortem samples, providing direct evidence of addiction-associated changes in gene expression in human subjects. Changes in gene experssion levels were also detected follwing binge cocaine self administration in rats. The identification of genes that are differentially expressed in subjects affected by addictive disorders provides methods for identifying genes and their expression products as targets in the diagnosis and treatment of addictive disorders. According to the invention, genes whose expression level is significantly increased or significantly decreased in subjects affected by an addictive disorder in comparison with control subjects are identified, along with their expression products, as screening targets for use in methods of identifying drugs for treating addictive disorders.

The methods of identifying genes whose expression is correlated with addiction provide an improvement in a method for screening for therapeutic compounds for use in treating addictive disorder. The improvement comprises the use of a target identified by the method described above.

The identification of genes whose expression is correlated with addiction is also in creating expression profiles correlated with addiction. Accordingly, in another aspect, the present invention provides methods for producing an expression profile having values representing the expression levels of genes whose expression is correlated with addictive disorders. The method comprises determining the expression level of one or more genes in a sample from subjects affected by an addictive disorder, determining the expression levels of the same genes in control subjects, and identifying genes whose expression level is significantly increased or significantly decreased in subjects affected by the addictive disorder in comparison with control subjects.

The invention also encompasses methods for predicting whether a compound will be addictive in humans. The methods comprise the steps of providing a reference expression profile associated with an addictive disorder, where the expression profile comprises values representing the expression level of one or more genes whose

expression is associated with the addictive disorder in subjects affected by the addictive disorder, providing a test expression profile comprising values representing the expression level of said one or more genes in a sample comprising cells that been contacted with the compound; and determining whether said test expression profile shares sufficient similarity to said reference profile. When the test expression profile is sufficiently similar to the reference profile, it is predicted that the compound will be addictive in humans.

The invention identifies genes that are differentially expressed in cocaine overdose victims in comparison with control subjects, and provides a method of screening for therapeutic compounds for use in treating addictive disorders using these genes. The method comprises screening for modulators of genes that are differentially expressed in cocaine overdose victims in comparison with control subjects.

In another aspect, the invention provides methods for evaluating a candidate drug to determine whether it will have therapeutic efficacy in treating an addictive disorder. The method comprises the steps of determining the expression level of one or more genes in a first sample from a subject affected by an addictive disorder prior to treatment with the candidate drug, wherein expression of said one or more genes is associated with the addictive disorder; determining the expression level of said one or more genes in a second sample from the subject following treatment with the candidate drug, and comparing the expression level measured before and after the treatment to determine the change in the expression level of said genes following treatment with the candidate drug. If the drug results in a change in the expression levels of the genes associated with the addictive disorder, it results in a prediction that the candidate drug will have therapeutic efficacy in treating the disorder.

Accordingly, the present invention provides methods for predicating the therapeutic efficacy and the potential for addiction of a candidate drug. By "therapeutic efficacy" it is intended the ability of the drug to alleviate (*e.g.*, mitigate, decrease, reduce) at least one of the symptom associated with the condition to be treated. By an "addictive" compound , it is intended a compound that creates a certain degree of euphoria in the subject and has a strong potential for addiction.

Compositions for use in the methods of the invention are also provided. Compositions of the invention include an array comprising a substrate having a

plurality of addresses, where each address has disposed thereon a capture probe that can specifically bind a nucleic acid molecule that is differentially expressed in subjects affected by cocaine addiction and a computer-readable medium having a digitally-encoded expression profile comprising one or more values representing the expression levels of a gene that is differentially expressed in cocaine overdose victims.

Compositions of the invention also include a kit for predicting whether a compound will be addictive in humans and a kit for evaluating the therapeutic efficacy of a candidate drug. The kits comprise an array having multiple addresses, wherein each address has a capture probe that can specifically bind a nucleic acid molecule that is differentially expressed in subjects affected by an addictive disorder; and a computer-readable medium having a digitally-encoded expression profiles having values representing the expression of a nucleic acid molecules detected by the array.

Expression Levels and Expression Profiles

As used herein, an "expression level" or "level of expression" is a value that corresponds to a measurement of the relative or absolute abundance of a gene expression product. Such values may include measurements of RNA levels or protein abundance. Thus, an expression level can be a value that reflects the transcriptional state or the translation state of a gene.

The transcriptional state of a sample includes the identities and abundance of the RNA species, especially mRNAs present in the sample. The transcriptional state can be conveniently determined by measuring transcript abundance by any of several existing gene expression technologies. Translational state includes the identities and abundance of the constituent protein species in the sample.

As used herein, an "expression profile" comprises one or more values corresponding to a measurement of the relative or absolute abundance of a gene expression product. Such values may include measurements of RNA levels or protein abundance. Thus, the expression profile can comprise values representing the measurement of the transcriptional state or the translational state of the gene. See, U.S. Pat. Nos. 6,040,138, 5,800,992, 6,020135, 6,344,316, and 6,033,860, which are hereby incorporated by reference in their entireties.

The transcriptional state of a sample includes the identities and relative abundance of the RNA species, especially mRNAs present in the sample. Preferably, a substantial fraction of all constituent RNA species in the sample are measured, but at least a sufficient fraction to characterize the transcriptional state of the sample is measured. The transcriptional state can be conveniently determined by measuring transcript abundance by any of several existing gene expression technologies. Translational state includes the identities and relative abundance of the constituent protein species in the sample.

In some embodiments, the expression profiles of the present invention are generated from samples from subjects affected by an addictive disorder. An "addictive disorder" as used herein is a disorder involving a pattern of compulsive drug use characterized by a continued craving for a drug and the need to use the drug for effects other than pain relief. Examples of addictive disorders of the present invention include, but are not limited to, addiction to cocaine, addiction to opiates (*e.g.*, heroin, methadone, morphine, oxycodone), addiction to stimulants (amphetamines, methamphetamine), hallucinogens (LSD, MDMA), delta-9-tetrahycrocannabinol (THC), alcohol, nicotine, benzodiazepines, and/or barbiturates. The samples from the subject affected by an addictive disorder used to generate the expression profiles of the present invention can be derived from a variety of sources including, but not limited to, single cells, a collection of cells, tissue, or cell culture. The tissue or cell source may include a post-mortem sample, cell culture, or a single cell.

In selecting a sample, the percentage of the sample that constitutes cells having differential gene expression in addictive disorders should be considered. Samples may comprise at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% cells having differential expression in addictive disorders, with a preference for samples having a higher percentage of such cells. In some embodiments, these cells are neuronal cells. In specific embodiments, these cells are ventral tegmental area cells or lateral substantia nigra cells. In some embodiments the sample is of human origin, while in other embodiments it is derived from a non-human model system for addictive disorders. Examples of model systems include rodent model (*e.g.* self- administration models, conditioned place preference models,

drug discrimination models, intracranial self-stimulation models, or locomoter activity models) and non-human primate models (*e.g.*, rhesus monkey, squirrel monkey, baboon) for self-administration or drug discrimination. Examples of such model systems are described, for example, in Hemby *et al.* (1997) Neuropharmcological basis of drug reinforcement, in *Drug Addiction and its Treatment: Nexus of Neuroscience and Behavior*, Johnson and Roache, Eds. Raven Press, NY, pp. 137-169); Hemby (1999) *Current Psychiatry Reports* 1:159-165; Koob and Goders (1989) Neuroanatomical substrates of drug self-administration, in *The Neuropharmcological Basis of Reward*, Liebman JM and Cooper SJ eds.) pp. 214-263, Clarendon Press, Oxford; Koob and Nester (1997) *Journal of Neuropsychiatry and Clinical Neurosciences* 9:482-497; and Wise and Bozarth (1987) A psychomotor stimulant theory of addiction. *Psychological Review* 94:469-492; each of which is herein incorporated by reference in its entirety.

The present invention also provides for measuring the level of gene in expression in control subjects. The control subjects of the invention are subjects that are not affected by drug addiction. In some embodiments, the control subjects matched to the test subjects affected by the addictive disorder by ensuring that both the control subject and the test subject are of a similar age. Where appropriate, other characteristics may also be matched between the control and test subjects such as race and sex. Matching characteristics of the test and control subjects reduces the likelihood that gene expression changes will results from a factor other than a difference in the use of addictive substances.

In some embodiments of the present invention, the expression profiles comprise values representing the expression levels of genes that are differentially expressed in subjects affected by an addictive disorder in comparison with control subjects. The term "differentially expressed" as used herein means that the measurement of a cellular constituent varies in two or more samples. The cellular constituent may be upregulated in a sample from a subject having one physiologic condition in comparison with a sample from a subject having a different physiologic condition, or down regulated in a sample from a subject having one physiologic condition in comparison with a sample from a subject having a different physiologic condition. Thus, the expression level of the gene may be significantly increased or significantly decreased in subjects affected by an addictive disorder in comparison with

control subjects. By a "significant" change in expression level, it is intended a change in expression level that is statistically significant. A statistical test may be used to test whether a change in expression level measured for a gene after treatment is more likely to result from an actual change in the expression of the gene rather than from any variability present in the experimental system.

The present invention provides groups of genes that are differentially expressed in cocaine overdose victims in comparison with age-matched control subjects. These genes were identified based on gene expression levels for 8700 probes in 8 post-mortem tissue samples from cocaine overdose victims and 8 post-mortem tissue samples from control subjects, and on mRNA and protein levels for candidate genes (Table 2). Values representing the expression levels of the nucleic acid molecules detected were analyzed to identify those that were significantly up- or down-regulated in cocaine overdose victims in comparison with control subjects. See the Experimental section.

The present provides genes with a differential level of expression in cocaine overdose victims compared to control subjects. These differentially expressed genes are selected from the genes shown in Tables 3, 4, and 5. These genes and their expression products are useful as markers to detect the presence of an addictive disorder in a patient.

The present also provides glutamate receptor subunits with a differential level of expression in rats following binge access to cocaine in comparison with controls. The genes encoding these differentially-expressed receptor subunits are shown in Table 6. These genes and their expression products are useful as markers to detect the presence of an addictive disorder in a patient, and as screening targets for compounds to treat addicitive disorders.

Each expression profile of the invention contains a sufficient number of values such that the profile can be used to distinguish samples correlated with addictive disorders from control samples. In some embodiments, the expression profiles comprise only one value. In other embodiments, the expression profile comprises more than one value corresponding to a differentially expressed gene, for example at least 2 values, at least 3 values, at least 4 values, at least 5 values, at least 6 values, at least 7 values, at least 8 values, at least 9 values, at least 10 values, at least 11 values, at least 12 values, at least 13 values, at least 14 values, at least 15 values, at least 16

values, at least 17 values, at least 18 values, at least 19 values, at least 20 values, at least 22 values, at least 25 values, at least 27 values, at least 30 values, at least 35 values , at least 40 values, at least 45 values, at least 50 values, at least 75 values, at least 100 values, at least 125 values, at least 150 values, at least 175 values, at least 200 values, at least 250 values, at least 300 values, at least 400 values, at least 500 values, at least 600 values, at least 700 values, at least 800 values, at least 900 values, at least 1000 values, at least 1200 values, at least 1500 values, or at least 2000 or more values.

It is recognized that the diagnostic accuracy of the methods of the present invention will vary based on the strength of the correlation between the expression levels of the differentially expressed genes and the associated physiologic condition. When the values in the expression profiles represent the expression levels of genes whose expression is strongly correlated with the physiologic condition, it may be possible to use fewer number of values in the expression profile and still obtain an acceptable level of diagnostic or prognostic accuracy.

By a gene whose expression level is "correlated with" a particular physiologic condition, it is intended a gene whose expression shows a statistically significant correlation with the physiologic condition. The significance of the correlation between the expression level of a differentially expressed gene and a particular physiologic state may be determined by a statistical test of significance. Such methods are known in the art and examples are provided elsewhere herein. Methods for determining the strength of a correlation between the expression level of a differentially-expressed gene and a particular physiologic state are also reviewed in Holloway et al. (2002) *Nature Genetics Suppl.* 32:481-89, Churchill (2002) *Nature Genetics Suppl.* 32:490-95, Quackenbush (2002) *Nature Genetics Suppl.* 32: 496-501; Slonim (2002) *Nature Genetics Suppl.* 32:502-08; and Chuaqui et al. (2002) *Nature Genetics Suppl.* 32:509-514; each of which is herein incorporated by reference in its entirety. Such methods may be used to select the genes whose expression levels have the greatest correlation with an addictive disorder in order to increase the predictive accuracy of the methods of the invention.

Each value in the expression profiles of the invention is a measurement representing the absolute or the relative expression level of a differentially expressed genes. The expression levels of these genes may be determined by any method known in the art for assessing the expression level of an RNA or protein molecule in a

sample. For example, expression levels of RNA may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are expressly incorporated herein by reference. The gene expression monitoring system may also comprise nucleic acid probes in solution.

In one embodiment of the invention, microarrays are used to measure the values to be included in the expression profiles. Microarrays are particularly well suited for this purpose because of the reproducibility between different experiments. DNA microarrays provide one method for the simultaneous measurement of the expression levels of large numbers of genes. Each array consists of a reproducible pattern of capture probes attached to a solid support. Labeled RNA or DNA is hybridized to complementary probes on the array and then detected by laser scanning. Hybridization intensities for each probe on the array are determined and converted to a quantitative value representing relative gene expression levels. See, the Experimental section. See also, U.S. Pat. Nos. 6,040,138, 5,800,992 and 6,020,135, 6,033,860, and 6,344,316, which are incorporated herein by reference. High-density oligonucleotide arrays are particularly useful for determining the gene expression profile for a large number of RNA's in a sample.

In one approach, total mRNA isolated from the sample is converted to labeled cRNA and then hybridized to an oligonucleotide array. Each sample is hybridized to a separate array. Relative transcript levels are calculated by reference to appropriate controls present on the array and in the sample. See, for example, the Experimental section.

In another embodiment, the values in the expression profile are obtained by measuring the abundance of the protein products of the differentially-expressed genes. The abundance of these protein products can be determined, for example, using antibodies specific for the protein products of the differentially-expressed genes. The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and

F(ab')2 fragments which can be generated by treating the antibody with an enzyme such as pepsin.

The antibody can be a polyclonal, monoclonal, recombinant, *e.g.*, a chimeric or humanized, fully human, non-human, *e.g.*, murine, or single chain antibody. In a preferred embodiment it has effector function and can fix complement. The antibody can be coupled to a toxin or imaging agent.

A full-length protein product from a differentially-expressed gene, or an antigenic peptide fragment of the protein product can be used as an immunogen. Preferred epitopes encompassed by the antigenic peptide are regions of the protein product of the differentially expressed gene that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity. The antibody can be used to detect the protein product of the differentially expressed gene in order to evaluate the abundance and pattern of expression of the protein. These antibodies can also be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given drug. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labeling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, b-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

Once the values comprised in the test expression profile and the reference expression profile or expression profiles are established, the subject profile is compared to the reference profile to determine whether the subject expression profile is sufficiently similar to the reference profile. Any method known in the art for comparing two or more data sets to detect similarity between them may be used to compare the subject expression profile to the reference expression profiles. To

determine whether a subject expression profile shows "statistically significant similarity" or "sufficient similarity" to a reference profile, statistical tests may be performed to determine whether the similarity between the subject expression profile and the reference expression profile is likely to have been achieved by a random event. Examples of such a statistical tests are described in the Experimental section; however, any statistical test that can calculate the likelihood that the similarity between the subject expression profile and the reference profile results from a random event can be used. The accuracy of assigning a subject to a risk group based on similarity between an expression profile for the subject and an expression profile for the risk group depends in part on the degree of similarity between the two profiles. Therefore, when more accurate diagnoses are required, the stringency with which the similarity between the subject expression profile and the reference profile is evaluated should be increased. For example, in various embodiments, the p-value obtained when comparing the subject expression profile to a reference profile that shares sufficient similarity with the subject expression profile is less than 0.20, less than 0.15, less than 0.10, less than 0.09, less than 0.08, less than 0.07, less than 0.06, less than 0.05, less than 0.04, less than 0.03, less than 0.02, or less than 0.01.

Arrays, Computer-Readable Medium, and Kits

The present invention provides compositions that are useful in the disclosed methods of identifying screening targets. These compositions include arrays comprising a substrate having a capture probes that can bind specifically to nucleic acid molecules that are differentially expressed in subjects affected by addictive disorders in comparison with control subjects. Also provided is a computer-readable medium having digitally encoded reference profiles useful in the methods of the claimed invention. The invention also encompasses kits comprising an array of the invention and a computer-readable medium having digitally-encoded reference profiles with values representing the expression of nucleic acid molecules detected by the arrays. These kits are useful for methods of evaluating the therapeutic efficacy of drugs for treating addictive disorders, and methods for predicting whether a compound will be addictive in humans.

The present invention provides arrays comprising capture probes for detecting the differentially expressed genes of the invention. By "array" is intended a solid

support or substrate with peptide or nucleic acid probes attached to said support or substrate. Arrays typically comprise a plurality of different nucleic acid or peptide capture probes that are coupled to a surface of a substrate in different, known locations. These arrays, also described as "microarrays" or colloquially "chips" have been generally described in the art, for example, in U.S. Patent. Nos. 5,143,854, 5,445,934, 5,744,305, 5,677,195, 6,040,193, 5,424,186, 6,329,143, and 6,309,831 and Fodor et al. (1991) Science 251:767-77, each of which is incorporated by reference in its entirety. These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods, which incorporate a combination of photolithographic methods and solid phase synthesis methods.

Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, *e.g.*, U.S. Patent No. 5,384,261, incorporated herein by reference in its entirety for all purposes. Although a planar array surface is preferred, the array may be fabricated on a surface of virtually any shape or even a multiplicity of surfaces. Arrays may be peptides or nucleic acids on beads, gels, polymeric surfaces, fibers such as fiber optics, glass or any other appropriate substrate, see U.S. Pat. Nos. 5,770,358, 5,789,162, 5,708,153, 6,040,193 and 5,800,992, each of which is hereby incorporated in its entirety for all purposes. Arrays may be packaged in such a manner as to allow for diagnostics or other manipulation of an all-inclusive device. See, for example, U.S. Pat. Nos. 5,856,174 and 5,922,591 herein incorporated by reference.

The arrays provided by the present invention comprise capture probes that can specifically bind a nucleic acid molecule that is differentially expressed in cocaine overdose victims. In some embodiments, each capture probe in the array detects a nucleic acid molecule selected from the nucleic acid molecules designated in Tables 3, 4, and 5. The designated nucleic acid molecules include those differentially expressed in subjects affected by addictive disorders in comparison with control subjects.

The arrays of the invention comprise a substrate have a plurality of addresses, where each addresses has a capture probe that can specifically bind a target nucleic acid molecule. The number of addresses on the substrate varies with the purpose for which the array is intended. The arrays may be low-density arrays or high-density arrays and may contain 4 or more, 8 or more, 12 or more, 16 or more, 20 or more, 24

or more, 32 or more, 48 or more, 64 or more, 72 or more 80 or more, 96, or more addresses, or 192 or more, 288 or more, 384 or more, 768 or more, 1536 or more, 3072 or more, 6144 or more, 9216 or more, 12288 or more, 15360 or more, or 18432 or more addresses. In some embodiments, the substrate has no more than 12, 24, 48, 96, or 192, or 384 addresses, no more than 500, 600, 700, 800, or 900 addresses, or no more than 1000, 1200, 1600, 2400, or 3600 addressees.

The invention also provides a computer-readable medium comprising one or more digitally-encoded expression profiles, where each profile has one or more values representing the expression of a gene that is differentially expressed in a subject affected by an addictive disorder. In some embodiments, the digitally-encoded expression profiles are comprised in a database. See, for example, U.S. Patent No. 6,308,170. The database may be screened to identify genes to be used as targets in a screen for compounds useful for treating addictive disorders.

The present invention also provides kits useful for evaluating the therapeutic efficacy or potential for addition of compounds or candidate drugs. These kits comprise an array and a computer readable medium. The array comprises a substrate having addresses, where each address has a capture probe that can specifically bind a nucleic acid molecule that is differentially expressed in subjects affected by an addictive disorder in comparison with control subjects. The results are converted into a computer-readable medium that has digitally-encoded expression profiles containing values representing the expression level of a nucleic acid molecule detected by the array.

Methods of Screening and Therapeutic Targets

The methods and compositions of the invention may be used to screen test compounds to identify therapeutic compounds useful for the treatment of addictive disorders. In one embodiment, the test compounds are screened in a sample comprising primary cells or a cell line representative of a neuronal cell type. After treatment with the test compound, the expression levels in the sample of one or more of the differentially-expressed genes of the invention are measured using methods described elsewhere herein. Values representing the expression levels of the differentially-expressed genes are used to generate a test expression profile. This subject expression profile is then compared to a reference profile associated with the

addictive disorder represented by the sample to determine the similarity between the subject expression profile and the reference expression profile. Differences between the subject expression profile and the reference expression profile may be used to determine whether the test compound has therapeutic efficacy in treating an addictive disorder.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in DeWitt et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al. (1994). *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop et al. (1994) *J. Med. Chem.* 37:1233. Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (U.S. Patent No. 5,223,409), plasmids (Cull et al. (1992) *Proc. Natl. Acad. Sci.* USA 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla et al. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 97:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310).

Candidate compounds include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries (see, *e.g.*, Lam et al. (1991) *Nature* 354:82-84; Houghten et al. (1991) *Nature* 354:84-86) and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids; 2) phosphopeptides (*e.g.*, members of random and partially degenerate, directed phosphopeptide libraries, see, *e.g.*, Songyang et al.

(1993) *Cell* 72:767-778); 3) antibodies (*e.g.*, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, F(ab¢)2, Fab expression library fragments, and epitope-binding fragments of antibodies); 4) small organic and inorganic molecules (*e.g.*, molecules obtained from combinatorial and natural product libraries; 5) zinc analogs; 6) leukotriene A4 and derivatives; 7) classical aminopeptidase inhibitors and derivatives of such inhibitors, such as bestatin and arphamenine A and B and derivatives; 8) and artificial peptide substrates and other substrates, such as those disclosed herein above and derivatives thereof.

The present invention discloses a number of genes that are differentially expressed in cocaine overdose victims in comparison with control subjects. These differentially-expressed genes are shown in Tables 3, 4, and 5. Because the expression of these genes is associated with drug addiction, these genes may play a role in the development of addiction. Accordingly, these genes and their gene products are potential therapeutic targets that are useful in methods of screening test compounds to identify therapeutic compounds for the treatment of addictive disorders.

The differentially-expressed genes of the invention may be used in cell-based screening assays involving recombinant host cells expressing the differentially-expressed gene product. The recombinant host cells are then screened to identify compounds that can activate the product of the differentially-expressed gene (i.e. agonists) or inactivate the product of the differentially-expressed gene (i.e. antagonists).

The following examples are offered by way of illustration and not intended to be limiting.

EXAMPLES

Example 1: Differential Expression of Candidate Genes in Cocaine Overdose Victims

A. Introduction

Chronic cocaine use in humans and animal models is known to lead to pronounced alterations in neuronal function in brain regions associated with drug reinforcement. To evaluate whether alterations in gene expression in cocaine overdose victims are associated with specific dopamine populations in the midbrain, cDNA arrays

and Western blotting were used to compare gene and protein expression patterns between cocaine overdose victims and age-matched controls in the ventral tegmental area (VTA) and lateral substantia nigra (l-SN). Array analysis revealed significant up-regulation of numerous transcripts in the VTA, but not l-SN, of cocaine overdose victims including NMDAR1, GluR2, GluR5 and KA2 receptor mRNA ($P<0.05$). No significant alterations between overdose victims and controls were observed for GluR1, R3 or R4 mRNA levels. Correspondingly, Western blot analysis revealed VTA-selective up-regulation of CREB ($P<0.01$) NMDAR1 ($P<0.01$), GluR2 ($P<0.05$), GluR5 ($P<0.01$), and KA2 ($P<0.05$) protein levels of cocaine overdose victims. The present results indicate that selective alterations of CREB and certain ionotropic glutamate receptor (iGluR) subtypes appear to be associated with chronic cocaine use in humans in a region specific manner. Moreover, since subunit composition determines the functional properties of iGluRs, the observed changes may indicate alterations in the excitability of dopamine transmission underlying long-term biochemical and behavioral effects of cocaine in humans.

Whereas animal models have advanced our understanding of the neurobiological basis of drug addiction, the evaluation of similar questions in human tissue are few, yet essential. Parallel investigations in human post-mortem tissue are important to determine whether biochemical changes observed and characterized in animal models are relevant to human drug abuse, as well as to identify novel changes that may be indicative of the human condition. The present study was undertaken to evaluate differences in gene expression in the VTA of cocaine overdose victims and age-matched, drug-free controls. In addition, we evaluated gene expression in the l-SN to provide a measure of brain region specificity. To this end, custom-designed macroarrays were used to simultaneously assess 81 genes and test the hypothesis that functional classes of genes were differentially expressed between cocaine overdose victims and controls in the VTA. Computational analyses were used to partition the data into groups of clones with similar function to facilitate interpretation of these data to the relevance of cocaine addiction. Select genes that were differentially expressed were evaluated at the protein level by Western blot analysis.

B. Experimental Procedures:

1. *Subjects and Tissue:*

Post-mortem human brain tissue was obtained at autopsy from 11 age-matched, drug-free control individuals (8WM, 1HM; 1BM; WF; Age: 35.0 ± 2.3 yrs., PMI=13.2 hrs ± 0.6 hrs) and 10 cocaine overdose victims (8WM, 2BM; Age: 35.5 ± 2.2 yrs., PMI=12.3 hrs ± 0.9 hrs) by the University of Miami Brain Endowment Bank (Table 1). For protein analysis, tissue punches were dissected from the VTA and l-SN of the contralateral hemisphere used for RNA analysis in eight cocaine overdose victims (34.4 ± 2.7 yr; 5WM, 3BM; PMI: 12.5 ± 1.1 hr) and seven age-matched, drug free controls (37 ± 2.7 yr; 6 WM, 1 WF; PMI: 12.6 ± 0.7 hr), representing a subset of subjects from whom tissue was used in the RNA experiments.

Table 1: Clinical Information of Study Subjects*

Subject	Race/ Gender	Age (yrs)	PMI (hrs)	Cause of Death	Toxi-cology	Blood		Brain		Cocethy-lene (Blood/ Brain)
						COC (mg/L)	BE (mg/L)	COC (mg/ kg)	BE (mg/ kg)	
COD1	W/M	39	12.5	Cocaine Intoxication	COC, CE	0.05	0.16	0.056	1.224	NA
COD2	W/M	36	13	Cocaine Intoxication; Athero-sclerotic Heart Disease	COC, CE, EtOH, LC	0.05	0.11	.136	.276	.204
COD3	W/M	41	12	Cocaine Intoxication; Cardiac Arrest	COC, EtOH	0.07	0.22	NA	NA	ND
COD4	B/M	32	18	Cocaine Intoxication	COC, NIC	0.2	.31	1.11	.89	
COD5	W/M	40	11.5	Cocaine Intoxication	COC, DPHA	0.05	.22	.119	<0.05	ND/ <0.05
COD6	B/M	34	14	Cocaine Intoxication	COC, ME	0.049	2.4			0.09/ND
COD7	W/M	41	12	Cocaine Intoxication	COC, ME	6.7	10	22.8	2.8	ND
COD8	W/M	23	8	Cocaine Intoxication	COC, ME	0.05	0.28	0.11	0.15	0.1/0.14
COD9	W/M	27	12	Cocaine Intoxication	COC			2.241	1.801	
COD10	W/M	42	10.5	Cocaine Intoxication	COC, CE	0.05	1.3	0.1	0.73	0.05/0.20
CTR1	W/M	35	16	Calcific Aortic Stenosis	ND					
CTR2	W/F	39	11	Sharp/Blunt Force Injuries	ND					
CTR3	W/M	44	11.5	Acute Myocardial Infarction	ND					

Table 1: Clinical Information of Study Subjects*

Subject	Race/ Gender	Age (yrs)	PMI (hrs)	Cause of Death	Toxi-cology	Blood		Brain		Cocethy-lene (Blood/ Brain)
						COC (mg/L)	BE (mg/L)	COC (mg/ kg)	BE (mg/ kg)	
CTR4	W/H/M	26	14	Idiopathic Cardiac Conduction System Disease	ND					
CTR5	W/M	24	15.5	Occlusive Coronary Artery Disease	ND					
CTR6	W/M	46	11	Aortic Aneurysim	ND					
CTR7	W/M	34	14	Coronary Arterio-sclerosis	ND					
CTR8	W/M	41	12	Athero-sclerotic Heart Disease	ND					
CTR9	W/M	34	11.5	Asthmatic Bronchitis	ND					
CTR10	W/M	37	14.5	NA	ND					
CTR11	B/M	33	14	Surgical Complica-tions	ND					

*Abbreviations: COC, cocaine; CE, cocethylene; COD: cocaine overdose; CTR: control; BE, benzylecognine; ND, not detectable; NA, not available; PMI, post-mortem interval; LC, lidocaine; EtOH, ethanol; DHPA: diphenhydramine

Gross and microscopic diagnostic neuropathologic examinations, which included examination of multiple cortical and subcortical regions, were performed in all cases and no neuropathological abnormalities relevant to mental status were found. All cases were retrospectively accrued based on toxicological data and circumstances surrounding the death, including review of prior arrest records and treatment admissions, as well as pathological indications (*e.g.*, perforation of the nasal septum, needle track marks) were reviewed carefully before classifying a cocaine intoxication case. All cases were evaluated for common drugs of abuse and alcohol, and positive urine screens were confirmed by quantitative analysis of blood. Cocaine and benzoylecgonine concentrations in brain and blood were assessed using gas chromatography/mass spectroscopy as described previously in Hernandez *et al.* (1994) *Forensic Science International* 65: 149-156, herein incorporated by reference in its entirety.

Following removal, brains were photographed and cut into 1.5 cm coronal blocks. Brain tissue was cryopreserved using a procedure described in Hardy *et al.* 1983

J. Neurochem. 40: 608-614; and Dodd *et al.* 1 *Neurochemical Pathology* 4, 177-198, both of which are herein incorporated by reference. One hundred mg punches were dissected from the blocks containing the VTA (posterior region of slab) and SN (anterior region containing the pars lateralis and the pars medialis) – one hemisphere for RNA and the contralateral hemisphere for protein analysis. Possible neuronal loss, ischemic cell changes, and reactive gliosis were assessed using semiquantitative ratings by the neuropathologists and found to be negligible in all cases used in the present study.

2. *RNA isolation and amplification:*

Micro-FastTrack® brand RNA isolation kits (Invitrogen; Carlsbad, California) were used to isolate polyadenylated RNA from the VTA and l-SN. mRNA yields ranged from 1 to 2 mg/100 ng of frozen tissue. Due to the amount of mRNA, samples from each subject were amplified using a modification of the aRNA amplification procedure described previously in Ginsberg *et al.* (2000) *Ann Neurol.* 48:77-87; Hemby *et al.* (2002) *Archives of General Psychiatry* 59:631-640; and Hemby *et al.* (2003) *Journal of Comparative Neurology* 456:176-183, each of which is herein incorporated by refrence in its entireity. Oligo(dT)-T7- primer/promoter (500 ng) [AAACGACGGCCAGTGAATTGTAAT ACGACTCACTATAGGCGC(T)24] (SEQ ID NO:1) was hybridized to poly(A+)mRNA in the presence of 500 mM each of dNTPS and RNase free water for 30 minutes at 65°C, then quick cooled on ice for 5 minutes. Next, 10 mM DTT, 1X first strand buffer and 20U RNAsin were added and samples were incubated at 42°C for 2 minutes as described in Van Gelder *et al.* (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:1663-1667. One ml of reverse transcriptase (Superscript™ II, RNase H-; Invitrogen; Carlsbad, California) was added to the solution and incubated at 42°C for 60 minutes. Following phenol/chloroform extraction and ethanol precipitation in the presence of 10 mg linear acrylamide, samples were re-suspended in RNase free water and heat denatured at 94°C for 5 minutes, then quick cooled. Next, 1X second strand buffer, 250 mM each of dNTPs, 10U Klenow fragment and 10U RNase H were added to the sample and incubated at 16°C for 4-6 hrs as described in Van Gelder *et al.* (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:1663-1667. At the end of the incubation, 2.5 mM b-nictotanimide adenine dinucleotide and 1000U E. coli DNA ligase were added to the solution and incubated at room temperature of 15 min, followed by the addition of 5U T4 DNA

polymerase at room temperature for 15 min as described in Sambrook and Russell (2001) *J. Neurosci.* 18:1848-1859. At the end of the incubation, 2.5 mM EDTA was added to stop the enzymatic reaction followed by phenol/chloroform extraction and ethanol precipitation. Following drop dialysis against RNase/DNase free water for 15 minutes, purified cDNA templates were used to generate antisense RNA (aRNA). aRNA was synthesized using T7 RNA polymerase (EpiCentre Technologies; Madison, WI) in 1X transcription buffer (Epicentre Technologies), 10 mM DTT, 250 mM each of NTPs and 20U RNAsin at 37°C overnight. Following phenol/chloroform extraction and ethanol precipitation, aRNA was reverse transcribed using Superscript II in the presence of 1X 1st strand buffer, 250 mM each of dNTPs, 100 ng of random hexamers and 20U RNAsin incubated at 37°C for one hr. For second strand cDNA synthesis, samples were incubated with 10U Klenow fragment, 250 mM each of dNTPs, 500 ng of the aforementioned oligo dT-T7 primer-promoter, and 5U T4 DNA polymerase at 16°C overnight. Samples were phenol/chloroform extracted, ethanol precipitated and drop dialyzed for 15 min.

For the second round of amplification, reaction conditions were identical for the first round except for the inclusion of 30 mCi [a-^{33}P]-UTP (2500 Ci/mmol; Amersham Pharmacia Biotech; Piscataway, NJ), 10 mM of UTP and 250 mM each of CTP, GTP and ATP. The aRNA procedure is a linear amplification process with minimal change in the relative abundance of the mRNA population in the native state of the neuron. mRNA can be reliably amplified from small amounts of fixed tissue including individual neurons and neuronal processes (Van Gelder *et al.* (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:1663-1667; Eberwine *et al.* (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89:3010-3014; Ginsberg *et al.* 1999 *Annals of Neurology* 45:174-181; Hemby *et al.* (2002) *Archives of General Psychiatry* 59:631-640; and Hemby *et al.* (2003) *Journal of Comparative Neurology* 456:176-183). Although there is a general decrease in mRNA levels in autopsied tissue, the relative abundance of gene expression remains unchanged (Castensson *et al.* (2000) *Genome Research* 10:1219-1229).

3. Macroarray Procedures

Reverse Northern Blots were prepared on nylon membranes containing candidate genes including dopamine receptors (*e.g.* D1, D2, D4, D5 and DAT), G-protein subunits (αi1, αi2, αs, αz, αq, αo, β, γ1 and γ2), glutamate receptor mRNAs

(mGluR3, GRIA1-4, GRIK5, 7 and GRIN1), GABA A receptor subunits (α1, α2, β1, β3, γ2, δ, ε, and π), regulators of G-protein signaling (RGS 1-7, 9, 10, 12, 13, 16) and other transcripts (cannabinoid 1 receptor, cocaine-amphetamine regulated transcript (CART), serotonin 2 and serotonin 3 receptors, and tyrosine hydroxylase). Inserts were amplified in 96 well plates using PCR with GF200 primers under the following conditions: 95°C for 5min (1 cycle); 95°C for 30 sec, 52°C for 45 sec, and 72°C for 2 min (40 cycles); and 72°C for 7 min (1 cycle). PCR samples were purified (Multiscreen™ PCR filter plate, Millipore, Bedford, MA) and aliquots were electrophoresed on a 1% agarose gel (1X TAE, 0.05% ethidium bromide) at 5V/cm for PCR band size verification. Gel images were captured by digital camera and archived. PCR product concentration was determined by spectrofluorometry (Gemini Microplate Spectrofluorometer, Molecular Devices, Sunnyvale, CA) using a 1:5000 dilution of SYBR 1 Green/TE and an aliquot of the PCR product. Values were compared to concentrations of known DNA standards for quantitation. 250 ng of each amplified insert was spotted on Nytran SuPerCharge ® nylon transfer membrane (Schleicher and Schuell, Keene, New Hampshire) using a 96 well dot blot apparatus (Schleicher and Schuell; Minifold I). DNA was crosslinked to the membrane by ultraviolet radiation.

Arrays were pre-hybridized with ULTRAhyb™ solution (Ambion; Austin, TX) in hybridization bottles for 1 hr at 42°C. Next, ^{33}P-labelled aRNA probes from each subject were heat denatured for 5 min at 65°C, and then immediately added to their respective bottles and allowed to hybridize for 18-24 hrs at 42°C in a rotisserie hybridization oven. Following hybridization, membranes were washed twice with 2X SSC/0.1% SDS and 0.1X SSC/0.1% SDS for 20 min each at 42°C. Labeled hybridized products were detected using phosphorimager screens, and hybridization signal intensities were analyzed using ImageQuant™ software (Amersham Biosciences; Sunnyvale, CA).

4. Protein preparation and Western blot analysis

Tissue samples were dounce homogenized in 10 mM HEPES, 10 mM NaCl, 1 mM KH2PO4, 5 mM $NaHCO_3$, 1mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM EDTA and the following protease inhibitors (PI): 1mM PMSF, 10 mM benzamidine, 10 mg/ml aprotinin, 10 mg/ml leupeptin, and 1 mg/ml pepstatin and centrifuged at 7500 RPM for 5 min. Supernatant was removed and the pellet (nuclei and debris) was resuspended in 20

mM Tris HCl, 1 mM EDTA (pH=8.0) with PIs and centrifuged at 7500 RPM for 5 min. This procedure was repeated twice and the pellet was resuspended in the solution and stored at -20°C (nuclear fraction). Supernatant was centrifuged at 25,000 RPM for 30 min at 4°C. Next, the supernatant containing the cytosolic fragment was removed and stored at -20°C (cytosolic fraction). The pellet was re-suspended in 10 mM Tris (pH=7.5), 300 mM sucrose, 1 mM EDTA (pH=8.0), 0.1% NP40 and PIs and centrifuged at 5000 RPM for 5 min at 4°C. The supernatant was discarded and the pellet was resuspended in the buffer and washed three times before re-suspension in the buffer and PIs and storing the samples at -20°C (membrane fraction).

Protein concentrations of samples were calculated using a bicinochoninic acid reagent protein assay kit (Pierce, Rockford, IL) and then the samples were diluted in Laemmli sample buffer to achieve the same final protein concentration. Identical amounts of proteins were loaded into a gel electrophoresis apparatus, subjected to sodium dodecyl sulfate-polyacrylamide (10%) gel electrophoresis (Bio-Rad, Richmond, CA) and transferred to nitrocellulose by electroblotting (30V, overnight at 4°C) in 1X transfer buffer (Bio-Rad). Nitrocellulose membranes were blocked in 0.5% nonfat dry milk and 0.1% Tween 20 in phosphate-buffered saline (pH 7.4, 0.12 M) for 1 hr at room temperature prior to being incubated with primary antibodies in blocking buffer (Bio-Rad) overnight at 4°C followed by secondary antibody for one hr at room temperature. Protein bands were visualized on a Kodak XAR-5 film with enhanced chemiluminescence (ECL Plus™, Amersham Pharmacia Biotech). Primary antibodies were as follows: mouse monoclonal antibodies directed against NMDAR1 (Chemicon International, Temecula, CA) and GluR5 (Upstate Biotechnologies, Waltham, MA); rabbit polyclonal antibodies directed against GluR1, GluR2/3, KA2, Gαs, Gαi1/2, Gβ, CREB, phospho-CREB (Upstate Biotechnologies) and FRA-2 (L-15; Santa Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies were HRP-conjugated anti-rabbit IgG and HRP-conjugated anti-mouse IgG (Upstate Biotechnologies). Protein abundances were calculated by optical densitometry with a Scan Jet 2200C and imported into NIH Image 1.61 software. Film background was subtracted from the optical density values to give the optical density value for a single subject. All assays were conducted under conditions in which densitometric signal intensity was linear with protein concentration as determined in preliminary experiments.

5. Data Analysis

Specific signal (minus background) of probe bound to each PCR product was expressed as a ratio of the total hybridization intensity of spots of the custom-designed array, thereby minimizing variations due to differences in the specific activity of the probe and the absolute quantity of probe present (Ginsberg *et al.* (2000) *Ann Neurol.* 48:77-87; Hemby *et al.* (2002) *Archives of General Psychiatry* 59:631-640; and Hemby *et al.* (2003) *Journal of Comparative Neurology* 456:176-183). Array data were analyzed using a two way ANOVA with group and anatomical region as the fixed effects and normalized hybridization intensity as the dependent measure. Post hoc analyses were conducted as needed using Tukey's Test and the null hypothesis was rejected when $P<0.05$. Western blot data were analyzed using t-tests comparing the band intensities between cocaine overdose victims and controls for each respective protein. Data were graphically depicted as percent of the controls for each respective protein. Null hypotheses were rejected when $P<0.05$.

C. Results

1. Subject data

There was no significant difference in age between cocaine overdose victims and age-matched, non-drug controls ($t=-0.156$, $df=19$, $P=0.877$), post-mortem interval ($t=0.847$, $df=19$, $P=0.408$), or brain pH ($t=0.431$, $df=17$, $P=0.672$) indicating these factors did not significantly influence the observed changes in gene or protein expression. Furthermore, neuroadaptive changes in the human brain post-mortem reflect chronic cocaine abuse, since death in a naive user is a rare occurrence, and the cohort of post-mortem subjects have many surrogate measures of chronicity (Ruttenber *et al.* 1997 *Journal of Forensic Science.* 42:25-31).

2. Gene Expression

Custom-designed macroarrays containing 81 cDNAs were used to evaluate gene expression changes in the VTA and l-SN of cocaine overdose victims and controls. The arrays contained a variety of transcripts including iGluR subunits (n=8), dopamine signaling (n=7), GABA transcripts (n=10), G-protein subunits (n=14), GTPase/RGS proteins (n=16), mRNA processing transcripts (n=8), cell growth/death transcripts (n=5), and others (n=13).

Receptors. There was a significant effect of cocaine overdose on glutamate receptor subunit mRNA expression in the VTA [F(1,209)=18.541; P<0.001] with a significant interaction between TRANSCRIPT and GROUP [F(1,9)=2.311; P<0.017]. Post hoc analysis revealed a significant increase in NMDAR1, GluR2, GluR5 and KA2, but not GluR1, GluR3 or GluR4 glutamate receptor subunits or the mGluR3 receptor mRNAs in the VTA of cocaine overdose victims (Table 2). mGluR3 and GluR3 were in low abundance in the VTA. Furthermore, no significant differences were observed in glutamate receptor mRNA expression in the l-SN between cocaine overdose victims and controls.

There was no significant difference in mRNA abundance for the various dopamine signaling transcripts including receptor subtypes (D1, D4, and D5), the dopamine transporter, tyrosine hydroxylase and dopa decarboxylase between cocaine overdose victims and controls in either the VTA [F(1, 146)=0.179, P=0.679] or l-SN [F(1,139)=0.135, P=0.714]. There was a trend towards significance in GABA signaling transcripts including GABA A receptor subunits (α1, α2, β1, β3, γ2, δ, ε, π) and glutamic acid decarboxylases (GAD) 65 and 67 in the VTA [F(1,230)=3.843, P=0.051]; however, there was no significant difference in these transcripts between the groups in the l-SN [F(1, 219)=1.980, P=0.161]. Analysis of other neurotransmitter signaling transcripts (CART, serotonin receptor subtype 2C and 3, and cannabinoid receptor 1) revealed a significant GROUP by TRANSCRIPT interaction [F(1,4)=4.440, P=0.003) that was attributable to a down-regulation of CART in the VTA of cocaine overdose victims compared to controls (P<0.05).

3. Signaling Cascade Transcripts

Examination of a variety of G-protein subunit mRNAs (Gα11, Gα15, Gαl, Gαi1, Gαi2, Gαs, Gαt, Gαz, Gαq, Gαo, Gβ1, and Gγ1-4) revealed a significant GROUP and TRANSCRIPT interaction [F(1,16)=1.863), P<0.023] in the VTA that was attributable to a down-regulation of the Gb1 subunit in cocaine overdose victims (P<0.05). Interestingly, there was a significant down-regulation of G-protein subunits in the l-SN of cocaine overdose victims compared to controls [F(1,339)=4.589, P<0.033], although there was no interaction.

GTPases and RGS proteins play an important role in regulating G protein function. In the VTA, there was a significant interaction of GROUP and TRANSCRIPT

for RGS/GTPase mRNAs (regulator of G-protein signaling (RGS) 1-7, 9, 10, 12, 13, 16, Rho guanine nucleotide exchange factor 5 (tim1), RAB7, member RAS oncogene family-like 1 (RAB7L1), and Ras homolog enriched in brain 2 (RHEB2)) [$F(1,314)=2.514$, $P=0.002$] that was attributable to a significant up-regulation of RGS3 ($P<0.05$) and down-regulation of RGS12 ($P<0.05$) in cocaine overdose victims. In contrast, there was a significant difference in the overall abundance of these transcripts between cocaine overdose victims and controls in the l-SN [$F(1,298)=4.951$, $P=0.027$], but no significant interaction (Table 2).

4. mRNA processing and cell growth/death related transcripts

Analysis of several transcript encoding proteins related to mRNA processing (fos-related antigen 2 (FRA2); early growth response (EGR) 2 and 3; protein kinase interferon-inducible double stranded RNA dependent (PRKR); CUG triplet repeat RNA binding protein 2 (CUGBP2); RNA polymerase II-DNA directed- polypeptide C 33kDa (POLR2C); suppressor of RNA polymerase B7 (RNApII); TATA box binding protein-associated factor, RNA polymerase II, H, 30kD (TBP-af30)) did not reveal any significant difference between cocaine overdose victims and controls in either the VTA or l-SN. Likewise, there was no significant difference in the abundance of cell growth/death related transcripts (activity-regulated cytoskeleton-associated protein (arc); brain derived neurotrophic factor (BDNF); growth associated protein 43 (GAP43); alpha spectrin, non-erythrocytic 1 (a-fodrin); and BCL2-associated X protein (bax)) between the groups in either the VTA or l-SN (Table 2).

Table 2: Statistical Analysis of Gene Expression Profiles for VTA and l-SN of Cocaine Overdose Victims*

Gene Class	Main Effect		Interaction		*Post Hoc* Analysis	
	VTA	l-SN	VTA	l-SN	VTA	l-SN
Glutamate Receptor	0.001	0.070	0.017	0.972		
GRIN (encoding NMDAR1)					0.033	-
GRIA1 (encoding GluR1)					-	-
GRIA2 (encoding GluR2)					0.002	-
GRIA3 (encoding GluR3)					-	-
GRIA4 (encoding GluR4)					-	-
GRIK1 (encoding GluR5)					0.003	-
GRIK5 (encoding KA2)					0.004	-
GRM3 (encoding mGluR3)					-	-
GABA transcripts	0.051	0.161	0.239	0.812		

Table 2: Statistical Analysis of Gene Expression Profiles for VTA and l-SN of Cocaine Overdose Victims*

Gene Class	Main Effect		Interaction		*Post Hoc* Analysis	
	VTA	l-SN	VTA	l-SN	VTA	l-SN
GABA subunit α1					-	-
GABA subunit α2					-	-
GABA subunit β1					-	-
GABA subunit β3					-	-
GABA subunit γ2					-	-
GABA subunit δ					-	-
GABA subunit ε					-	-
GABA subunit νπ					-	-
GAD65					-	-
GAD67					-	-
G-protein subunits	0.590	0.033	0.023	1.000		
Gαi1					-	-
Gαi2					-	-
Gαo					-	-
Gαl					-	-
Gαs					-	-
Gαt					-	-
Gαq					-	-
Gαz					-	-
Gα11					-	-
Gα15					-	-
Gβ1					0.001	-
Gγ1					-	-
Gγ2					-	-
Gγ4					-	-
Dopamine transcripts	0.673	0.714	0.531	0.760		
D1 receptor					-	-
D2 receptor					-	-
D4 receptor					-	-
D5 receptor					-	-
Dopamine transporter					-	-
Tyrosine hydroxylase					-	-
Dopa decaroxylase					-	-
Regulators of G protein signaling (RGS) & GTPases	0.175	0.027	0.002	1.000	-	-
RGS1					-	-
RGS2					-	-
RGS3					0.001	-
RGS4					-	-
RGS5					-	-
RGS6					-	-
RGS7					-	-
RGS9					-	-
RGS10					-	-
RGS11					-	-
RGS12					0.002	-
RGS13					-	-
RGS16					-	-
Rho guanine nucleotide exchange factor (GEF) 5					-	-
RAB7, member RAS					-	-

Table 2: Statistical Analysis of Gene Expression Profiles for VTA and l-SN of Cocaine Overdose Victims*

Gene Class	Main Effect		Interaction		*Post Hoc* Analysis	
	VTA	l-SN	VTA	l-SN	VTA	l-SN
oncogene family-like 1 Ras homolog enriched in brain 2					-	-
mRNA processing Fos related antigen 2 Early growth response 2 Early growth response 3 protein kinase, interferon-inducible RNA dependent CUG triplet repeat, RNA binding protein 2 RNApII TATA box binding protein-associated factor, 30kDa RNA polymerase II polypeptide C, 33kDa	0.204	0.210	0.442	0.998	- - - - - - -	- - - - - - -
Cell growth/death activity-regulated cytoskeleton-associated protein (arc) Brain derived neurotrophic factor growth associated protein 43 (GAP43); neuromodulin α fodrin BCL2-associated X protein (bax)	0.759	0.515	0.118	0.943	- - - - -	- - - - -
Miscellaneous Cocaine-amphetamine related transcript 5-hydroxytryptamine (serotonin) receptor 2A 5-hydroxytryptamine (serotonin) receptor 2C 5-hydroxytryptamine (serotonin) receptor 3 Cannabinoid receptor 1	0.481	0.278	0.003	0.205	0.001 - - - -	- - - - -

Table 3: Genes from Table 2 showing significant regulation in cocaine overdose victims	
3A: Genes up-regulated in cocaine overdose victims	
Gene Name	*Accession Number*
GRIN (encoding NMDAR1)	R88267
GRIA2 (encoding GluR2)	H28734
GRIK1 (encoding GluR5)	R44776
GRIK5 (encoding KA2)	NM_002088
RGS3	AI369623
3B: Genes down-regulated in cocain overdose victims	
Gene Name	*Accession Number*
G-protein subunit Gβ1	R24969
RGS12	NM_002926
Cocaine-amphetamine related transcript	AI300511

5. Protein expression.

Western blot analysis was performed on protein homogenates from individual subjects to assess the correlation of changes in mRNA levels with protein levels. From the gene expression analysis, proteins for NR1, GluR1, GluR2, GluR5, KA2, Gαi1, Gαs, and Gβ were selected based on the availability of selective antibodies. In addition, protein levels of cAMP responsive element binding protein (CREB), phosphorylated CREB, and FRA2 were evaluated based on their regulation in animal models of drug abuse (Carlezon *et al.* (1998) *Science* 282:2272-2275; Self *et al.* (1998) *Journal of Neuroscience* 18:1848-1859; Pliakas *et al.* (2001) *Journal of Neuroscience* 21:7397-7403).

In membrane fractions, NR1 immunoreactive protein was increased 111.2% ($P<0.05$), GluR2/3 by 69.1% ($P<0.05$), GluR5 by 507.9% ($P<0.01$), and KA2 by 72.1% ($P<0.05$) in the VTA of cocaine overdose victims, whereas GluR1 showed a non-significant 50.3% increase in this region. Comparatively, these proteins were slightly increased in the l-SN, albeit in a non-significant manner (NR1: 12.9%; GluR1: 56.1%; GluR2/3: 30.1%; GluR5: 13.4%; KA2: 50.4%). A previous study indicates that the aforementioned iGluR subunits are stable up to 18 hr PMI (Wang *et al.* (2000) *Molecular Brain Research* 80:123-131), suggesting that the present results are not compromised by proteolysis.

Protein levels were increased 23.3% for Gαi1/2 and 23.6% for Gαs while Gβ levels were decreased 21.7% in the VTA of cocaine overdose victims compared to controls in cytosolic fragments. In the l-SN, Gαi1/2 was increased by 10%, whereas

Gαs was decreased by 15.1% and Gβ 10.1% in cocaine overdose victims compared to controls, all in a statistically non-significant manner.

Previous studies have hypothesized that adaptive changes induced by cocaine on the cAMP intracellular cascade can alter subsequent responsiveness of the drug (Carlezon *et al.* (1998) *Science* 282:2272-2275; Self *et al.* (1998) *Journal of Neuroscience* 18:1848-1859; Pliakas *et al.* (2001) *Journal of Neuroscience* 21:7397-7403). Evaluation of CREB immunoreactive protein revealed a significant up-regulation (69.6%; $P<0.05$) of CREB in the cytosolic fraction from the VTA, but not l-SN, of cocaine overdose victims. There was a slight but statistically non-significant increase in CREB immunoreactivity in the nuclear protein fraction from the VTA (34.3%), whereas levels in the l-SN were similar between cocaine overdose victims and controls. Phospho-CREB immunoreactivity was not detectable in either the cytosolic or nuclear fractions in either brain regions of cocaine overdose victims or controls (data not shown). There was no significant difference in FRA-2 protein levels between cocaine overdose victims and controls in either the VTA or l-SN.

D. Conclusions

The results described above demonstrate the up-regulation of NR1, GluR2, GluR5 and KA2 glutamate receptor subunit mRNA and protein levels specific to the VTA in human cocaine overdose victims. Previous studies in rodents indicated that upregulation of iGluR subunits was associated with augmented dopamine-glutamate interactions in the mesolimbic pathway (White *et al.* (1995) *Journal of Pharmacology & Experimental Therapeutics* 273:445-454; White *et al.* (1995) *Neurophysiological alterations in the mesocorticolimbic doapmine system during cocaine administration*, in *The Neurobiology of Cocaine Addiction*, Hammer R. P., Jr., ed. pp 99-120, CRC Press, Boca Raton, FL; Zhang *et al.* (1997) *Journal of Pharmacology & Experimental Therapeutics* 281:699-706; White and Kalivas (1998) *Drug & Alcohol Dependence* 51:141-153; Wolf (1998); Vanderschuren and Kalivas (2000) *Psychopharmacologia* 151:99-120; and Giorgetti *et al.* (2001) *Journal of Neuroscience* 21:6362-6369. The present invention demonstrates that increased NR1 protein levels in cocaine overdose victims may be induced by acute or sub-acute cocaine exposure or possibly reflect the last acute cocaine binge prior to death. Upregulation of mRNA and protein levels of

GluR2, GluR5 and KA2 are indicative of chronic cocaine use and/or cocaine overdose in humans.

In the VTA and SN, GluR1-4 and NR1 immunoreactivity was found to be localized almost exclusively on dopaminergic neurons (Paquet *et al.* 1997 *J. Neurosci.* 17:1377-1396); however, less is known about the localization of kainate subunits in these regions. Glutamatergic afferents in the midbrain originate from the medial frontal cortex, subthalamic and pedunculopontine tegmental nuclei (Christie *et al.* (1985) *Brain Research* 333:169-172.; Sesack *et al.* (1989) *Journal of Comparative Neurology* 290:213-242; Sesack and Pickel (1992) *Journal of Comparative Neurology* 320:145-160; and Lu *et al.* (1997) . *Synapse* 25:205-214. A recent study suggested prefrontal glutamatergic projections selectively synapse on mesoprefrontal dopamine neurons and mesoaccumbens GABA neurons in the VTA in rats (Carr and Sesack (2000); *Journal of Neuroscience* 20:3864-3873, although the topography in non-human primates and humans remains unknown. The presence of these subunits on dopamine neurons and the upregulation reported in the present study may provide a means by which to influence dopamine excitability in this region. Upregulation of the kainite preferring subunits GluR5 and KA2 in cocaine overdose victims and the probable localization of these subunits on dopamine neurons represent additional means by which increased Ca2+ influx may lead to hyperexcitability of VTA dopamine neurons. Interestingly, GluR2 subunit protein levels were increased in the hippocampus of alcoholics (Breese et al. (1995) *Brain Research* 674:82-90) and GluR5 mRNA and protein levels were increased in the dorsal prefrontal cortex of cocaine treated rats (Toda *et al.* (2002) *Journal of Neurochemistry* 82, 1290-1299). Since extensive editing of GluR2 in the adult brain renders ionophores less permeable to Ca2+, the upregulation observed in the present study may represent a molecular compensation for the effects of increased Ca2+ influx generated by the combined upregulation of NR1, GluR5 and KA2 (Paschen *et al.* (1994) *Journal of Neurochemistry.* 63:1596-1602). In summary, although the present invention is not bound by a particular mechanism, upregulation of the iGluR subunit mRNA and protein levels in the present study are likely attributable to changes in VTA dopaminergic neurons, suggesting a neuroadaptive response to cocaine in these subjects.

Dopamine receptor mRNAs were not differentially regulated as a function of cocaine overdose, opening the possibility that intracellular signaling mechanisms may also contribute to the altered function of these neurons. Neuroadaptations in the cAMP

pathway have been implicated in mesolimbic brain regions as a function of cocaine exposure (Nestler *et al.* (1990) *Journal of Neurochemistry*. 55:1079-1082; Terwilliger *et al.* (1991) *Brain Research*. 548:100-110; Striplin and Kalivas (1992) *Brain Research*. 579:181-186; Miserendino and Nestler (1995) *Brain Research*. 674:299-306; Carlezon *et al.* (1998) Brain Research. 674:299-306; Self *et al.* (1998) *Journal of Neuroscience*. 18:1848-1859; Pliakas *et al.* (2001) *Journal of Neuroscience*. 21:7397-7403). Assessment of various α, β, and γ G-protein subunits revealed only a significant decrease in Gβ1 subunit mRNA, but not protein, in the VTA of cocaine overdose victims. The discrepancy between Gβ1 mRNA and protein levels may be reflective of post-transcriptional, translational, or post-translational processing/degradation and/or trafficking of the protein outside of the regions studied. In addition, decreased Gβ mRNA levels may manifest as down-regulated protein levels in axonal targets of the mesoaccumbens dopamine neurons as shown previously in cocaine-treated rats (Wang *et al.* (1997) *Journal of Neuroscience* 17:5993-6000. The present data contrast previous studies showing decreased ADP ribosylation and immunoreactivity Gαi and Gαo in the VTA of cocaine treated rats. However, a significant increase in CREB mRNA and protein levels in the cytosolic fraction and a trend towards significance in the nuclear fraction in the VTA of cocaine overdose victims was observed - the first demonstration of increased CREB protein levels in the VTA of either humans or animal models as a function of cocaine exposure. The length of the post mortem intervals for these subjects likely contributed to the inability to detect phosphorylated CREB imunoreactivity; precluding speculation on the role of elevated CREB levels in the present study. Although CREB is almost exclusively expressed in the nucleus, CREB protein has been identified in the cytoplasm by light and electron microscopy (Ferrer *et al.* (1996) *Journal of Neuroscience* 16:274-282; Hermanson *et al.* (1996) *Biochemical & Biophysical Research Communications* 225:256-262 ; Suzuki *et al.* (1998) *Molecular Brain Research*. 61:69-77; Shaywitz and Greenberg (1999) *Annual Review of Biochemistry* 68:821-861) as well as in post-synaptic density fractions (Suzuki *et al.* (1998) *Molecular Brain Research*. 61:69-77) and dendrites (Crino *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95:2313-2318) leading to the hypothesis of nuclear translocation as a means of site-specific plasticity (Crino *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95:2313-2318; Suzuki *et al.* (1998) *Molecular Brain Research*. 61:69-77).

In the present study, statistical analysis indicated a trend towards significance in nuclear CREB ($P<0.07$) in the VTA of cocaine overdose victims. In addition, the ability of the antibody to detect the antigen in the nucleus may be impaired by conformational changes in the protein due to dimerization, binding of the dimer to DNA, and/or masking of the antigen by CREB binding proteins or other transcriptional regulators. A more precise role of cytoplasmic CREB and the mechanism underlying elevated CREB levels remains to be determined in animal models of the disease. The relevance of altered CREB levels in the present study supports several studies in rodents which indicated CREB levels were inversely proportional to the rewarding effects of the drug (Carlezon *et al.* (1998) *Science.* 282:2272-2275; Walters and Blendy (2001) *Journal of Neuroscience* 21:9438-9444.. One manner in which CREB activity may be regulated is by Ca2+ influx via NMDA receptors, demonstrating a requirement for CREB-NMDA receptor interactions in dopamine-regulated gene expression (Konradi et al. (1996) *Journal of Neuroscience* 16, 4231-4239).

One of the transcripts regulated by CREB is CART (Dominguez *et al.* (2002) *Journal of Neurochemistry* 80:885-893). Discovered as a novel transcript in the striatum whose regulation was induced by acute cocaine (Douglass *et al.* (1995) *Journal of Neuroscience* 15:2471-2481), CART peptides were shown to be abundant in the NAc, VTA and SN as well as other brain regions (Smith *et al.* (1997) *Synapse* 27:90-94; Smith *et al.* (1999) *Journal of Comparative Neu*rology 407:491-511; Hurd and Fagergren (2000) *Journal of Comparative Neurology* 425:583-598; and Dallvechia-Adams *et al.* (2002) *Journal of Comparative Neurology* 448:360-372. The present study is the first to demonstrate CART mRNA regulation by chronic cocaine use in humans.

The upregulation of RGS3 and RGS 12 mRNAs in the VTA of cocaine overdose victims represents another potential mechanism for cocaine-induced alterations in cell signaling. Previous studies have shown elevated RGS 2, 3 and 5 mRNA levels in the striatum by acute amphetamine administration (Burchett *et al.* (1999) *Journal of Neurochemistry* 72:1529-1533) and RGS 2 mRNA levels by cocaine in the hippocampus, cortex, and striatum (Ingi *et al.* (1998) *Journal of Neuroscience* 18:7178-7188). RGS proteins modulate/integrate G-protein and other intracellular signaling proteins, some of which reduce the duration of G-protein activation by increasing the rate of GTP hydrolysis. In vivo, RGS3 interacts with the G protein bg complex to accelerate the rate of recovery of N-type Ca2+ inhibition from Gbg thereby leading to

increased Ca2+ conductance and potentially to increased excitability of VTA dopamine neurons.

Example 2: Microarray Analysis of Molecular Correlates of Cocaine Addiction

The present example describes the identification of genes that are differentially expressed in the VTA of cocaine overdose victims in comparison with age-matched controls. Gene expression from the VTA of cocaine overdose victims (n=8) and age-matched controls (n=8) using high-density microarrays (UniGem V. 2.0, Incyte Pharmaceuticals, Inc) having capture probes for 8700 human transcripts. mRNA from each subject was isolated as described above in Example 1. Equivalent concentrations of mRNA from each subject within the cocaine overdose group or the control group were combined. Pooled samples were reverse transcribed and hybridized to the microarrays, and the resulting image was analyzed to determine gene expression levels. Pooling samples decreases the influence of individual variability and stresses the identification of those transcripts that are most highly regulated in the sample population. Hierarchical (degree of differential expression) and functional (protein classes, functional pathways, etc.) clustering of the data reveals a subset of genes that were assessed further by subsequent reverse Northern analysis. Using 1.8-fold differential expression as a cut-off for the hierarchical analysis, greater than 250 transcripts are down-regulated (Table 4) and greater than 1800 transcripts are up-regulated (Table 5) in the VTA from cocaine overdose victims.

Table 4: Transcripts down-regulated in cocaine overdose victims.

Gene Expression Ratio	GeneName	CloneID	Accession Number
-5.2	zinc finger protein 267 {Incyte PD: 3117417}	3117417	X78925
-4.2	zinc finger protein 175 {Incyte PD: 1306335}	1306335	D50419
-3.8	phospholipase A2, group IB (pancreas) {Incyte PD: 2383830}	2383830	NM_000928
-3.5	zinc finger protein 184 (Kruppel-like) {Incyte PD: 2837773}	2837773	AL021918
-3.1	transcription factor 17 {Incyte PD: 3316844}	3316844	NM_005649
-3	MADS box transcription enhancer factor 2, polypeptide D (myocyte enhancer factor 2D) {Incyte PD: 2722916}	2722916	NM_005920
-3	zinc finger protein 7 (KOX 4, clone HF.16) {Incyte PD: 1858215}	1858215	M29580
-2.8	carnitine palmitoyltransferase II {Incyte PD: 1225508}	1225508	NM_000098
-2.7	ESTs, Weakly similar to !!!! ALU SUBFAMILY SQ WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 2242122}	2242122	AA074214

Gene Expression Ratio	GeneName	CloneID	Accession Number
-2.6	cerebellar degeneration-related protein (34kD) {Incyte PD: 382416}	382416	NM_004065
-2.6	coatomer protein complex, subunit alpha {Incyte PD: 3296228}	3296228	AL043506
-2.6	RAB7, member RAS oncogene family-like 1 {Incyte PD: 829449}	829449	D84488
-2.5	ATPase, H+ transporting, lysosomal (vacuolar proton pump), beta polypeptide, 56/58kD, isoform 1 {Incyte PD: 2676425}	2676425	M25809
-2.5	ESTs {Incyte PD: 2952124}	2952124	AA993264
-2.5	Incyte EST {Incyte PD: 2879384}	2879384	W38850
-2.5	oligophrenin 1 {Incyte PD: 4216520}	4216520	AJ001189
-2.4	ESTs {Incyte PD: 1431969}	1431969	AA481492
-2.4	ESTs {Incyte PD: 2855084}	2855084	N33584
-2.4	general transcription factor IIH, polypeptide 2 (44kD subunit) {Incyte PD: 1352669}	1352669	AW148977
-2.4	Incyte EST {Incyte PD: 3097582}	3097582	AW341105
-2.4	neurogranin (protein kinase C substrate, RC3) {Incyte PD: 1943863}	1943863	Y15058
-2.4	thyroid stimulating hormone receptor {Incyte PD: 1336836}	1336836	M32215
-2.4	tumor necrosis factor (ligand) superfamily, member 14 {Incyte PD: 2512776}	2512776	AF064090
-2.4	zinc finger protein 273 {Incyte PD: 600744}	600744	X78932
-2.4	zinc finger protein 38 (KOX 25) {Incyte PD: 997067}	997067	AA481221
-2.4	zinc finger protein 45 (a Kruppel-associated box (KRAB) domain polypeptide) {Incyte PD: 2909710}	2909710	L75847
-2.3	EST {Incyte PD: 2707290}	2707290	N62143
-2.3	ESTs {Incyte PD: 1760693}	1760693	AA455180
-2.3	ESTs {Incyte PD: 2881469}	2881469	W88829
-2.3	ESTs {Incyte PD: 3127513}	3127513	AA769438
-2.3	G protein-coupled receptor 64 {Incyte PD: 2728317}	2728317	X81892
-2.3	histone deacetylase 3 {Incyte PD: 1805745}	1805745	AF039703
-2.3	Incyte EST {Incyte PD: 2793857}	2793857	AW341105
-2.3	KIAA0321 protein {Incyte PD: 2907827}	2907827	AB002319
-2.3	peptidylprolyl isomerase (cyclophilin)-like 2 {Incyte PD: 3600608}	3600608	U37219
-2.3	transcription factor 7 (T-cell specific, HMG-box) {Incyte PD: 1877423}	1877423	X59871
-2.2	ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide {Incyte PD: 1622542}	1622542	J05096
-2.2	ATP-binding cassette, sub-family D (ALD), member 4 {Incyte PD: 2904720}	2904720	Y14318
-2.2	class I cytokine receptor {Incyte PD: 1659090}	1659090	AF053004
-2.2	ESTs {Incyte PD: 1404401}	1404401	N24233
-2.2	ESTs {Incyte PD: 2136337}	2136337	AA506165
-2.2	ESTs {Incyte PD: 2149058}	2149058	AA858162
-2.2	ESTs {Incyte PD: 3822328}	3822328	AI655142
-2.2	ESTs {Incyte PD: 567779}	567779	AA056332
-2.2	Incyte EST {Incyte PD: 2447550}	2447550	AL528044
-2.2	Incyte EST {Incyte PD: 2674482}	2674482	H48664
-2.2	Incyte EST {Incyte PD: 2896792}	2896792	

Gene Expression Ratio	GeneName	CloneID	Accession Number
-2.2	Incyte EST {Incyte PD: 399433}	399433	X78926
-2.2	KIAA0419 gene product {Incyte PD: 3268220}	3268220	AI110758
-2.2	Kruppel-type zinc finger (C2H2) {Incyte PD: 2580149}	2580149	AB011414
-2.2	LIM protein (similar to rat protein kinase C-binding enigma) {Incyte PD: 2791283}	2791283	NM_006457
-2.2	lymphocyte antigen 75 {Incyte PD: 2554240}	2554240	AF011333
-2.2	MAD (mothers against decapentaplegic, Drosophila) homolog 4 {Incyte PD: 2705026}	2705026	NM_005359
-2.2	matrix metalloproteinase-like 1 {Incyte PD: 3031706}	3031706	NM_004142
-2.2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100) {Incyte PD: 994439}	994439	S76638
-2.2	nucleoporin 62kD {Incyte PD: 3181360}	3181360	AA323842
-2.2	PRP4/STK/WD splicing factor {Incyte PD: 2671453}	2671453	NM_004697
-2.2	PTPRF interacting protein, binding protein 1 (liprin beta 1) {Incyte PD: 1957118}	1957118	AA459628
-2.2	TATA box binding protein (TBP)-associated factor, RNA polymerase I, A, 48kD {Incyte PD: 3211615}	3211615	AA311701
-2.2	T-cell receptor, alpha (V,D,J,C) {Incyte PD: 2917006}	2917006	X64643
-2.2	zinc finger protein 266 {Incyte PD: 619699}	619699	X78924
-2.1	cathepsin S {Incyte PD: 2768610}	2768610	M90696
-2.1	chitobiase, di-N-acetyl- {Incyte PD: 2879077}	2879077	AI148751
-2.1	deoxyribonuclease II, lysosomal {Incyte PD: 1675345}	1675345	AF060222
-2.1	dihydrofolate reductase {Incyte PD: 134850}	134850	W03282
-2.1	DKFZP586A0522 protein {Incyte PD: 1965049}	1965049	H53438
-2.1	EGF-like-domain, multiple 4 {Incyte PD: 2311825}	2311825	AB011541
-2.1	ESTs {Incyte PD: 1399652}	1399652	AA620873
-2.1	ESTs {Incyte PD: 1740678}	1740678	F12749
-2.1	ESTs {Incyte PD: 1928644}	1928644	AA557277
-2.1	ESTs {Incyte PD: 2472778}	2472778	W85702
-2.1	ESTs {Incyte PD: 2556708}	2556708	AI308112
-2.1	ESTs {Incyte PD: 3044552}	3044552	AI374654
-2.1	ESTs {Incyte PD: 3121962}	3121962	H81066
-2.1	ESTs {Incyte PD: 4215852}	4215852	AA700126
-2.1	ESTs, Weakly similar to alternatively spliced product using exon 13A [H.sapiens] {Incyte PD: 3244710}	3244710	AI816116
-2.1	ESTs, Weakly similar to similar to genome polyprotein [C.elegans] {Incyte PD: 2917949}	2917949	AA460131
-2.1	ESTs, Weakly similar to Zn-finger-like protein [H.sapiens] {Incyte PD: 3293122}	3293122	AI202783
-2.1	histidine triad nucleotide-binding protein {Incyte PD: 2827453}	2827453	W02839
-2.1	Homo sapiens clone 24507 mRNA sequence {Incyte PD: 3773032}	3773032	AF052148
-2.1	Homo sapiens iduronate-2-sulfatase (IDS) mRNA, complete cds {Incyte PD: 2776385}	2776385	AF050145
-2.1	Incyte EST {Incyte PD: 2045705}	2045705	
-2.1	Incyte EST {Incyte PD: 2160566}	2160566	AI676236
-2.1	Incyte EST {Incyte PD: 3335669}	3335669	
-2.1	interleukin 6 receptor {Incyte PD: 873406}	873406	X12830
-2.1	KIAA0414 protein {Incyte PD: 1977267}	1977267	AB007874
-2.1	KIAA0575 gene product {Incyte PD: 3094261}	3094261	AB011147

Gene Expression Ratio	GeneName	CloneID	Accession Number
-2.1	KIAA0576 protein {Incyte PD: 1812489}	1812489	AB011148
-2.1	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1 {Incyte PD: 2747113}	2747113	AF009007
-2.1	myosin IB {Incyte PD: 2474755}	2474755	X98507
-2.1	neuropeptide Y receptor Y6 {Incyte PD: 3875782}	3875782	NM_006173
-2.1	nucleolar autoantigen (55kD) similar to rat synaptonemal complex protein {Incyte PD: 81490}	81490	U47621
-2.1	potassium inwardly-rectifying channel,subfamily J, member 13 {Incyte PD: 1849449}	1849449	AJ006128
-2.1	sialyltransferase 8 (alpha-2, 8-polysialytransferase) D {Incyte PD: 706712}	706712	L41680
-2.1	signal transducer and activator of transcription 3 (acute-phase response factor) {Incyte PD: 487291}	487291	AJ012463
-2.1	surfactant, pulmonary-associated protein B {Incyte PD: 1988674}	1988674	J02761
-2.1	transcription factor 7 (T-cell specific, HMG-box) {Incyte PD: 2655513}	2655513	NM_003202
-2.1	tumor protein, translationally-controlled 1 {Incyte PD: 3027978}	3027978	AI719469
-2.1	v-crk avian sarcoma virus CT10 oncogene homolog {Incyte PD: 1858909}	1858909	AL043540
-2.1	zinc finger protein 44 (KOX 7) {Incyte PD: 2398861}	2398861	X16281
-2	ATP-binding cassette, sub-family F (GCN20), member 2 {Incyte PD: 2185537}	2185537	AL050291
-2	branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease) {Incyte PD: 1759220}	1759220	NM_000056
-2	calmodulin 2 (phosphorylase kinase, delta) {Incyte PD: 2803306}	2803306	D45887
-2	caspase 2, apoptosis-related cysteine protease (neural precursor cell expressed, developmentally down-regulated 2) {Incyte PD: 162793}	162793	U13022
-2	coronin, actin-binding protein, 2A {Incyte PD: 2210264}	2210264	U57057
-2	COX15 (yeast) homolog, cytochrome c oxidase assembly protein {Incyte PD: 1976440}	1976440	AF044323
-2	DNA fragmentation factor, 45 kD, alpha subunit {Incyte PD: 1415990}	1415990	NM_004401
-2	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 {Incyte PD: 3493645}	3493645	NM_004432
-2	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked) {Incyte PD: 671776}	671776	M61733
-2	EST {Incyte PD: 3278047}	3278047	AA909818
-2	EST {Incyte PD: 3714075}	3714075	AA772405
-2	ESTs {Incyte PD: 1968436}	1968436	C14608
-2	ESTs {Incyte PD: 2689747}	2689747	AW118969
-2	ESTs {Incyte PD: 2749278}	2749278	AA431300
-2	ESTs {Incyte PD: 2818928}	2818928	AA219088
-2	eukaryotic translation initiation factor 3, subunit 2 (beta, 36kD) {Incyte PD: 2054115}	2054115	AA306891
-2	GATA-binding protein 2 {Incyte PD: 1569804}	1569804	M68891
-2	gene near HD on 4p16.3 with homology to hypothetical S. pombe gene {Incyte PD: 997717}	997717	AB000467
-2	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme {Incyte PD: 1966455}	1966455	Z19550

Gene Expression Ratio	GeneName	CloneID	Accession Number
-2	glutamate-cysteine ligase (gamma-glutamylcysteine synthetase), regulatory (30.8kD) {Incyte PD: 132212}	132212	NM_002061
-2	H.sapiens mRNA for XIAP associated factor-1 {Incyte PD: 4738521}	4738521	X99699
-2	Homo sapiens clone 23676 mRNA sequence {Incyte PD: 4093234}	4093234	AF035278
-2	Incyte EST {Incyte PD: 2418337}	2418337	AI584082
-2	Incyte EST {Incyte PD: 2478920}	2478920	
-2	Incyte EST {Incyte PD: 2589593}	2589593	AW502401
-2	interferon-induced protein with tetratricopeptide repeats 4 {Incyte PD: 2840251}	2840251	AF026939
-2	interleukin 10 {Incyte PD: 1747050}	1747050	M57627
-2	interleukin 17 receptor {Incyte PD: 2365149}	2365149	U58917
-2	KIAA0365 gene product {Incyte PD: 1900378}	1900378	AB002363
-2	KIAA0759 protein {Incyte PD: 1319195}	1319195	AI338315
-2	MAD (mothers against decapentaplegic, Drosophila) homolog 5 {Incyte PD: 87235}	87235	AF009678
-2	minichromosome maintenance deficient (S. cerevisiae) 4 {Incyte PD: 103669}	103669	AW135046
-2	mitogen-activated protein kinase 12 {Incyte PD: 3267303}	3267303	U66243
-2	myelin-associated oligodendrocyte basic protein {Incyte PD: 1486348}	1486348	H23197
-2	no arches-like (zebrafish) zinc finger protein; CLEAVAGE-POLYADENYLATION SPECIFICITY FACTOR, 30-KD {Incyte PD: 1919968}	1919968	AA203670
-2	p300/CBP-associated factor {Incyte PD: 1622953}	1622953	NM_003884
-2	pericentriolar material 1 {Incyte PD: 777175}	777175	H00568
-2	phosphorylase kinase, gamma 1 (muscle) {Incyte PD: 2952043}	2952043	X80590
-2	platelet/endothelial cell adhesion molecule (CD31 antigen) {Incyte PD: 887867}	887867	M37780
-2	primase, polypeptide 1 (49kD) {Incyte PD: 105121}	105121	NM_000946
-2	proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated) {Incyte PD: 1485846}	1485846	M27110
-2	sialyltransferase 4A (beta-galactosidase alpha-2,3-sialytransferase) {Incyte PD: 2926307}	2926307	L29555
-2	small inducible cytokine subfamily A (Cys-Cys), member 22 {Incyte PD: 4589052}	4589052	AC004382
-2	sperm surface protein {Incyte PD: 2004896}	2004896	AI241173
-2	synovial sarcoma, translocated to X chromosome {Incyte PD: 1671035}	1671035	X79201
-2	TATA box binding protein (TBP)-associated factor, RNA polymerase II, A, 250kD {Incyte PD: 617467}	617467	D90359
-2	teratocarcinoma-derived growth factor 1 {Incyte PD: 1275388}	1275388	X14253
-2	vasoactive intestinal peptide receptor 2 {Incyte PD: 1596589}	1596589	NM_003382
-2	zinc finger protein 33a (KOX 31) {Incyte PD: 3209530}	3209530	D31763
-1.9	apoptosis inhibitor 2 {Incyte PD: 1513214}	1513214	U37546
-1.9	calcium channel, voltage-dependent, alpha 2/delta subunit 2 {Incyte PD: 3642720}	3642720	AF042793
-1.9	carboxypeptidase M {Incyte PD: 1525881}	1525881	NM_001874
-1.9	caspase 6, apoptosis-related cysteine protease {Incyte PD: 3615147}	3615147	NM_001226

Gene Expression Ratio	GeneName	CloneID	Accession Number
-1.9	CMRF35 leukocyte immunoglobulin-like receptor {Incyte PD: 4413820}	4413820	NM_006678
-1.9	endothelial cell growth factor 1 (platelet-derived) {Incyte PD: 2473910}	2473910	S72487
-1.9	ESTs {Incyte PD: 1366043}	1366043	W74700
-1.9	ESTs {Incyte PD: 1414220}	1414220	AA781074
-1.9	ESTs {Incyte PD: 1425861}	1425861	AA004443
-1.9	ESTs {Incyte PD: 2845586}	2845586	AA527277
-1.9	ESTs {Incyte PD: 2917169}	2917169	AW271626
-1.9	ESTs {Incyte PD: 2968970}	2968970	D20764
-1.9	ESTs {Incyte PD: 3563809}	3563809	AA682865
-1.9	ESTs {Incyte PD: 465591}	465591	AA156287
-1.9	ESTs {Incyte PD: 546616}	546616	N52088
-1.9	ESTs {Incyte PD: 623166}	623166	H49213
-1.9	ESTs, Highly similar to ACF7 protein [H.sapiens] {Incyte PD: 2246234}	2246234	AI017174
-1.9	ESTs, Moderately similar to !!!! ALU SUBFAMILY J WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 520342}	520342	AA354153
-1.9	ESTs, Moderately similar to !!!! ALU SUBFAMILY SB2 WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 2156769}	2156769	AW081018
-1.9	ESTs, Moderately similar to !!!! ALU SUBFAMILY SP WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 1911371}	1911371	AA700925
-1.9	ESTs, Weakly similar to !!!! ALU SUBFAMILY J WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 2039995}	2039995	R10357
-1.9	ESTs, Weakly similar to !!!! ALU SUBFAMILY SC WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 2458029}	2458029	R98130
-1.9	forkhead box O3A {Incyte PD: 1581467}	1581467	AF032886
-1.9	G protein-coupled receptor 4 {Incyte PD: 1518613}	1518613	NM_005282
-1.9	heterogeneous nuclear ribonucleoprotein A0 {Incyte PD: 637639}	637639	U23803
-1.9	Homo sapiens clone 23728 mRNA sequence {Incyte PD: 2209136}	2209136	AF038199
-1.9	Homo sapiens mRNA, chromosome 1 specific transcript KIAA0492 {Incyte PD: 1732133}	1732133	AB007961
-1.9	Homo sapiens mRNA, chromosome 1 specific transcript KIAA0495 {Incyte PD: 1963554}	1963554	AI806400
-1.9	Human BRCA2 region, mRNA sequence CG006 {Incyte PD: 4240806}	4240806	U50535
-1.9	huntingtin (Huntington disease) {Incyte PD: 3293711}	3293711	T03830
-1.9	Incyte EST {Incyte PD: 1794858}	1794858	
-1.9	Incyte EST {Incyte PD: 2131862}	2131862	NM_000511
-1.9	Incyte EST {Incyte PD: 2820372}	2820372	
-1.9	Incyte EST {Incyte PD: 3080656}	3080656	
-1.9	inositol 1,4,5-triphosphate receptor, type 3 {Incyte PD: 2790678}	2790678	NM_002224
-1.9	integral membrane protein 2B {Incyte PD: 3096030}	3096030	AW131784
-1.9	interferon (alpha, beta and omega) receptor 1 {Incyte PD: 3564703}	3564703	X60459
-1.9	interleukin 11 {Incyte PD: 3733572}	3733572	NM_000881
-1.9	jerky (mouse) homolog {Incyte PD: 5159418}	5159418	AF072467
-1.9	KIAA0063 gene product {Incyte PD: 3227603}	3227603	D31884
-1.9	KIAA0159 gene product {Incyte PD: 495967}	495967	D63880

Gene Expression Ratio	GeneName	CloneID	Accession Number
-1.9	KIAA0524 protein {Incyte PD: 2612729}	2612729	AB011096
-1.9	KIAA0738 gene product {Incyte PD: 547281}	547281	AB018281
-1.9	melanoma adhesion molecule {Incyte PD: 1994460}	1994460	AF089868
-1.9	paraoxonase 2 {Incyte PD: 2703308}	2703308	T96910
-1.9	phosphotidylinositol transfer protein, beta {Incyte PD: 2820503}	2820503	AL035843
-1.9	potassium channel, subfamily K, member 3 (TASK) {Incyte PD: 2370491}	2370491	AI193606
-1.9	RNA polymerase II transcriptional regulation mediator (Med6, S. cerevisiae, homolog of) {Incyte PD: 1633994}	1633994	AA305926
-1.9	solute carrier family 31 (copper transporters), member 1 {Incyte PD: 2176211}	2176211	NM_001859
-1.9	stearoyl-CoA desaturase (delta-9-desaturase) {Incyte PD: 2845102}	2845102	AA357347
-1.9	T54 protein {Incyte PD: 155678}	155678	AW250209
-1.9	tuberous sclerosis 2 {Incyte PD: 1634046}	1634046	AC005600
-1.9	tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator) {Incyte PD: 2121653}	2121653	NM_003820
-1.9	tumor necrosis factor receptor superfamily, member 7 {Incyte PD: 1870589}	1870589	AA831844
-1.9	zinc finger protein 165 {Incyte PD: 1689436}	1689436	U78722
-1.9	zinc finger protein 184 (Kruppel-like) {Incyte PD: 4198926}	4198926	AL021918
-1.9	zinc finger protein 230 {Incyte PD: 3344757}	3344757	NM_006300
-1.9	zinc finger protein 272 {Incyte PD: 4310558}	4310558	X78931
-1.9	zinc finger, X-linked, duplicated A {Incyte PD: 1485817}	1485817	AL034396
-1.8	acetyl LDL receptor; SREC {Incyte PD: 2783668}	2783668	AI689097
-1.8	choroideremia (Rab escort protein 1) {Incyte PD: 3354893}	3354893	AL022401
-1.8	coagulation factor II (thrombin) receptor-like 1 {Incyte PD: 1801553}	1801553	Z49993
-1.8	colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage) {Incyte PD: 86947}	86947	X17648
-1.8	cubilin (intrinsic factor-cobalamin receptor) {Incyte PD: 4335824}	4335824	NM_001081
-1.8	DKFZP586J0917 protein {Incyte PD: 1968721}	1968721	AL117455
-1.8	DNA segment, single copy, probe pH4 (transforming sequence, thyroid-1, {Incyte PD: 3043293}	3043293	S72869
-1.8	dynamin 1 {Incyte PD: 1942557}	1942557	L07810
-1.8	ESTs {Incyte PD: 1602726}	1602726	AA814948
-1.8	ESTs {Incyte PD: 1709249}	1709249	N37065
-1.8	ESTs {Incyte PD: 1880426}	1880426	W52566
-1.8	ESTs {Incyte PD: 2174773}	2174773	AI281113
-1.8	ESTs {Incyte PD: 2359637}	2359637	AW194419
-1.8	ESTs {Incyte PD: 2404172}	2404172	AI088364
-1.8	ESTs {Incyte PD: 2608167}	2608167	AW003228
-1.8	ESTs {Incyte PD: 2950228}	2950228	AI082610
-1.8	ESTs {Incyte PD: 993050}	993050	AI263389
-1.8	ESTs, Highly similar to !!!! ALU SUBFAMILY SX WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 1849453}	1849453	N93536
-1.8	ESTs, Moderately similar to KIAA0909 protein [H.sapiens] {Incyte PD: 1984396}	1984396	D58019
-1.8	ESTs, Moderately similar to recombination activating gene 1 inducing protein [H.sapiens] {Incyte PD: 334820}	334820	X84712

Gene Expression Ratio	GeneName	CloneID	Accession Number
-1.8	ESTs, Moderately similar to rhotekin [M.musculus] {Incyte PD: 1383182}	1383182	AI498180
-1.8	ESTs, Weakly similar to Na/PO4 cotransporter homolog [H.sapiens] {Incyte PD: 2296344}	2296344	AA258357
-1.8	GM2 ganglioside activator protein {Incyte PD: 1526212}	1526212	X62078
-1.8	homeodomain-interacting protein kinase 3 {Incyte PD: 2725511}	2725511	AF004849
-1.8	Homo sapiens chromosome 19, cosmid R29368 {Incyte PD: 4592475}	4592475	AC004262
-1.8	Homo sapiens mRNA for inositol 1,4,5-trisphosphate 3-kinase isoenzyme, partial cds {Incyte PD: 1313615}	1313615	D38169
-1.8	Homo sapiens mRNA; cDNA DKFZp564D0462 (from clone DKFZp564D0462) {Incyte PD: 1227602}	1227602	T80121
-1.8	Human acidic 82 kDa protein mRNA, complete cds {Incyte PD: 1453445}	1453445	U15552
-1.8	Human clone 23933 mRNA sequence {Incyte PD: 2286572}	2286572	U79273
-1.8	Human DNA sequence from PAC 127B20 on chromosome 22q11.2-qter, contains gene for GTPase-activating protein similar to rhoGAP protein. ribosomal protein L6 pseudogene, ESTs and CA repeat {Incyte PD: 2044444}	2044444	Z83838
-1.8	Incyte EST {Incyte PD: 1461515}	1461515	
-1.8	Incyte EST {Incyte PD: 1602194}	1602194	
-1.8	Incyte EST {Incyte PD: 3176179}	3176179	NM_001251
-1.8	interleukin enhancer binding factor 1 {Incyte PD: 1622587}	1622587	U58196
-1.8	KIAA0118 protein {Incyte PD: 1802745}	1802745	D42087
-1.8	KIAA0544 protein {Incyte PD: 4174315}	4174315	AB011116
-1.8	KIAA0638 protein {Incyte PD: 684589}	684589	AB014538
-1.8	kinesin family member 3B {Incyte PD: 1659338}	1659338	NM_004798
-1.8	leptin (murine obesity homolog) {Incyte PD: 1961822}	1961822	NM_000230
-1.8	LINE retrotransposable element 1 {Incyte PD: 3421817}	3421817	AL021937
-1.8	myosin IB {Incyte PD: 1985354}	1985354	X98507
-1.8	nucleoporin 62kD {Incyte PD: 2085746}	2085746	T08684
-1.8	pancreatic lipase-related protein 1 {Incyte PD: 2084515}	2084515	NM_006229
-1.8	PBX/knotted 1 homeobox 1 {Incyte PD: 2374272}	2374272	Y13613
-1.8	peptidylprolyl isomerase D (cyclophilin D) {Incyte PD: 2073720}	2073720	NM_005038
-1.8	pericentrin {Incyte PD: 1655365}	1655365	AB007862
-1.8	postmeiotic segregation increased 2-like 6 {Incyte PD: 623240}	623240	D38503
-1.8	potassium inwardly-rectifying channel, subfamily J, member 3 {Incyte PD: 2555034}	2555034	U39196
-1.8	pre-mRNA splicing factor SF3a (60kD), similar to S. cerevisiae PRP9 (spliceosome-associated protein 61) {Incyte PD: 1329895}	1329895	U08815
-1.8	RAB interacting factor {Incyte PD: 1645179}	1645179	U74324
-1.8	RAB2, member RAS oncogene family {Incyte PD: 2842857}	2842857	AI458986
-1.8	ribosomal protein, mitochondrial, S12 {Incyte PD: 1946178}	1946178	AF058761
-1.8	RNA polymerase II transcriptional regulation mediator (Med6, S. cerevisiae, homolog of) {Incyte PD: 2844031}	2844031	AF074723
-1.8	solute carrier family 15 (oligopeptide transporter), member 1 {Incyte PD: 3325119}	3325119	NM_005073
-1.8	solute carrier family 6 (neurotransmitter transporter, GABA),	3244361	X54673

Gene Expression Ratio	GeneName	CloneID	Accession Number
	member 1 {Incyte PD: 3244361}		
-1.8	sudD (suppressor of bimD6, Aspergillus nidulans) homolog {Incyte PD: 1730689}	1730689	NM_003831
-1.8	TLS-associated serine-arginine protein {Incyte PD: 1515905}	1515905	AW057530
-1.8	translin-associated factor X {Incyte PD: 3357245}	3357245	NM_005999
-1.8	trinucleotide repeat containing 12 {Incyte PD: 2739049}	2739049	U80743
-1.8	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3 {Incyte PD: 1698713}	1698713	M29366
-1.8	zinc finger protein 197 {Incyte PD: 2811412}	2811412	AF011573
-1.8	zinc finger protein 91 (HPF7, HTF10) {Incyte PD: 3493687}	3493687	L11672

Table 5: Transcripts up-regulated in cocaine overdose victims.

Gene Expression Ratio	GeneName	CloneID	Accession Number
6.2	CUG triplet repeat,RNA-binding protein 2 {Incyte PD: 2056288}	2056288	AF036956
5.6	klotho {Incyte PD: 3322706}	3322706	AB005142
5.1	ESTs {Incyte PD: 797919}	797919	AI826495
4.6	thyroid peroxidase {Incyte PD: 2875604}	2875604	J02969
3.6	chemokine (C-C motif) receptor 1 {Incyte PD: 1874307}	1874307	D10925
3.4	ESTs {Incyte PD: 2785471}	2785471	AI091432
3.4	KIAA0902 protein {Incyte PD: 1834526}	1834526	AB020709
3.4	nuclear receptor coactivator 1 {Incyte PD: 939126}	939126	AA495962
3.2	CD8 antigen, alpha polypeptide (p32) {Incyte PD: 1846142}	1846142	M12824
3.1	aquaporin 9 {Incyte PD: 3752047}	3752047	AB008775
3.1	ESTs {Incyte PD: 2678945}	2678945	AI457221
3	ESTs {Incyte PD: 2803551}	2803551	AA976778
3	ESTs {Incyte PD: 3039213}	3039213	AI522241
3	Homo sapiens PAC clone DJ1093O17 from 7q11.23-q21 {Incyte PD: 4955989}	4955989	AA333314
2.9	collagen, type X, alpha 1 (Schmid metaphyseal chondrodysplasia) {Incyte PD: 4855492}	4855492	X98568
2.9	ESTs {Incyte PD: 1889561}	1889561	AW050488
2.9	ESTs {Incyte PD: 2767042}	2767042	AA504591
2.9	fibronectin leucine rich transmembrane protein 2 {Incyte PD: 4286401}	4286401	AB007865
2.9	Homo sapiens clone 24739 mRNA sequence {Incyte PD: 4984469}	4984469	AF070571
2.9	membrane-spanning 4-domains, subfamily A, member 3 (hematopoietic cell-specific) {Incyte PD: 1831058}	1831058	L35848
2.9	PET112 (yeast homolog)-like {Incyte PD: 2366650}	2366650	AA448188
2.9	phospholipase A2 receptor 1, 180kD {Incyte PD: 1401683}	1401683	U17033
2.8	ESTs {Incyte PD: 1287278}	1287278	N92790
2.8	ESTs {Incyte PD: 1403294}	1403294	AI133467
2.8	ESTs {Incyte PD: 1845509}	1845509	AI288887
2.8	ESTs {Incyte PD: 1868612}	1868612	AA085721

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.8	Homo sapiens RAG2 mRNA, partial cds {Incyte PD: 2936676}	2936676	AW058148
2.8	phosphoinositide-3-kinase, class 2, gamma polypeptide {Incyte PD: 2762837}	2762837	AJ000008
2.8	Protein inhibitor of activated STAT X {Incyte PD: 1981629}	1981629	N25685
2.7	amphiregulin (schwannoma-derived growth factor) {Incyte PD: 2352645}	2352645	NM_001657
2.7	angiotensin receptor 2 {Incyte PD: 3607580}	3607580	U20860
2.7	ATP-binding cassette, sub-family B (MDR/TAP), member 4 {Incyte PD: 4285457}	4285457	M23234
2.7	calcitonin-related polypeptide, beta {Incyte PD: 2847188}	2847188	AI937230
2.7	casein, alpha {Incyte PD: 3479268}	3479268	NM_001890
2.7	chromosome-associated polypeptide C {Incyte PD: 2160794}	2160794	NM_005496
2.7	cystathionase (cystathionine gamma-lyase) {Incyte PD: 666190}	666190	S52784
2.7	desmoglein 1 {Incyte PD: 3561805}	3561805	AF097935
2.7	dTDP-D-glucose 4,6-dehydratase {Incyte PD: 2373504}	2373504	AI972505
2.7	ESTs {Incyte PD: 1406708}	1406708	AI768720
2.7	ESTs {Incyte PD: 1724856}	1724856	AA203219
2.7	high-mobility group (nonhistone chromosomal) protein isoform I-C {Incyte PD: 1446475}	1446475	U28749
2.7	macrophage scavenger receptor 1 {Incyte PD: 3943651}	3943651	D13264
2.7	membrane component, chromosomal 4, surface marker (35kD glycoprotein) {Incyte PD: 2816509}	2816509	M32306
2.7	protease inhibitor 5 (maspin) {Incyte PD: 1628341}	1628341	AI435384
2.7	protein kinase, cGMP-dependent, type II {Incyte PD: 2232413}	2232413	X94612
2.7	sperm associated antigen 6 {Incyte PD: 2360811}	2360811	AL080136
2.7	TBP-interacting protein {Incyte PD: 1842689}	1842689	AA971779
2.7	tryptophan 2,3-dioxygenase {Incyte PD: 2769155}	2769155	NM_005651
2.6	ATP binding protein associated with cell differentiation {Incyte PD: 1804666}	1804666	AB006679
2.6	carnitine/acylcarnitine translocase {Incyte PD: 1857756}	1857756	NM_000387
2.6	chymotrypsin-like protease {Incyte PD: 1464211}	1464211	NM_001907
2.6	collagen, type IX, alpha 1 {Incyte PD: 2748931}	2748931	AW022764
2.6	downregulated in ovarian cancer 1 {Incyte PD: 1977073}	1977073	U53445
2.6	estrogen receptor 1 {Incyte PD: 4116386}	4116386	X03635
2.6	ESTs {Incyte PD: 1398814}	1398814	N41458
2.6	ESTs {Incyte PD: 1603857}	1603857	AI417860
2.6	ESTs {Incyte PD: 1639638}	1639638	AW316760
2.6	ESTs {Incyte PD: 1640094}	1640094	AA654772
2.6	ESTs {Incyte PD: 1866866}	1866866	AI278685
2.6	ESTs {Incyte PD: 2207102}	2207102	N39074
2.6	ESTs {Incyte PD: 2960859}	2960859	AA928141
2.6	ESTs, Moderately similar to CDV-1R protein [M.musculus] {Incyte PD: 1494531}	1494531	AI277889
2.6	ESTs, Weakly similar to 25 kDa trypsin inhibitor [H.sapiens] {Incyte PD: 2121278}	2121278	T77033
2.6	Homo sapiens HRIHFB2017 mRNA, partial cds {Incyte PD: 4142721}	4142721	AA625489
2.6	KIAA0008 gene product {Incyte PD: 1970111}	1970111	D13633

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.6	KIAA0125 gene product {Incyte PD: 3220430}	3220430	D50915
2.6	KIAA0831 protein {Incyte PD: 1560627}	1560627	AI921835
2.6	mutS (E. coli) homolog 4 {Incyte PD: 5325961}	5325961	NM_002440
2.6	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C) {Incyte PD: 1406825}	1406825	AI767296
2.6	nuclear receptor interacting protein 1 {Incyte PD: 2366468}	2366468	X84373
2.6	protein S (alpha) {Incyte PD: 2592991}	2592991	X12892
2.6	retinoblastoma-like 1 (p107) {Incyte PD: 1513664}	1513664	L14812
2.6	solute carrier family 26 (sulfate transporter), member 2 {Incyte PD: 1424624}	1424624	U14528
2.6	Thyrotropin receptor {3' region} [human, mRNA Partial, 1573 nt] {Incyte PD: 1441542}	1441542	S82807
2.5	3-prime-phosphoadenosine 5-prime-phosphosulfate synthase 2 {Incyte PD: 1603408}	1603408	NM_004670
2.5	AND-1 protein {Incyte PD: 3032613}	3032613	AJ006266
2.5	ATP-binding cassette, sub-family B (MDR/TAP), member 10 {Incyte PD: 1725719}	1725719	N58275
2.5	bullous pemphigoid antigen 1 (230/240kD) {Incyte PD: 1922531}	1922531	M69225
2.5	catenin (cadherin-associated protein), alpha-like 1 {Incyte PD: 2379808}	2379808	AF080071
2.5	deleted in azoospermia {Incyte PD: 912500}	912500	AA608617
2.5	ESTs {Incyte PD: 1663925}	1663925	AW135567
2.5	ESTs {Incyte PD: 1753033}	1753033	AA406526
2.5	ESTs {Incyte PD: 1817969}	1817969	AA488929
2.5	ESTs {Incyte PD: 2041712}	2041712	AA280957
2.5	ESTs {Incyte PD: 2122324}	2122324	N95414
2.5	ESTs {Incyte PD: 2291537}	2291537	AA875999
2.5	ESTs {Incyte PD: 2364159}	2364159	AA779691
2.5	ESTs {Incyte PD: 2459904}	2459904	AI373658
2.5	ESTs {Incyte PD: 2752460}	2752460	N59757
2.5	ESTs {Incyte PD: 2754640}	2754640	AA971264
2.5	ESTs {Incyte PD: 2806295}	2806295	AA394082
2.5	ESTs {Incyte PD: 3409027}	3409027	AI805082
2.5	ESTs {Incyte PD: 3427271}	3427271	AI459175
2.5	ESTs {Incyte PD: 606593}	606593	AA687278
2.5	ESTs {Incyte PD: 757370}	757370	AI760485
2.5	ESTs, Moderately similar to nuclear receptor co-repressor N-CoR [H.sapiens] {Incyte PD: 1274084}	1274084	AA468619
2.5	ESTs, Weakly similar to 6-PYRUVOYL TETRAHYDROBIOPTERIN SYNTHASE [H.sapiens] {Incyte PD: 2586533}	2586533	AW023933
2.5	ESTs, Weakly similar to CGI-82 protein [H.sapiens] {Incyte PD: 2717861}	2717861	AI309334
2.5	glypican 4 {Incyte PD: 2182642}	2182642	AA887423
2.5	H.sapiens novel gene from PAC 117P20, chromosome 1 {Incyte PD: 960406}	960406	AA668786
2.5	Incyte EST {Incyte PD: 2026332}	2026332	
2.5	Incyte EST {Incyte PD: 2045819}	2045819	AI246762
2.5	Incyte EST {Incyte PD: 2046989}	2046989	BC005868
2.5	KIAA0175 gene product {Incyte PD: 3805046}	3805046	D79997

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.5	KIAA0849 protein {Incyte PD: 4203430}	4203430	AI362018
2.5	lymphoid-restricted membrane protein {Incyte PD: 2060553}	2060553	NM_006152
2.5	matrix metalloproteinase 13 (collagenase 3) {Incyte PD: 1949348}	1949348	NM_002427
2.5	matrix metalloproteinase 19 {Incyte PD: 1532028}	1532028	U37791
2.5	MCF.2 cell line derived transforming sequence {Incyte PD: 1373037}	1373037	X12556
2.5	Meis (mouse) homolog 2 {Incyte PD: 1252255}	1252255	AF017418
2.5	mitogen-activated protein kinase kinase 3 {Incyte PD: 2244069}	2244069	AI950268
2.5	multimerin {Incyte PD: 781087}	781087	U27109
2.5	myeloid cell nuclear differentiation antigen {Incyte PD: 633460}	633460	M81750
2.5	nuclear transcription factor, X-box binding 1 {Incyte PD: 2989547}	2989547	U15306
2.5	osteoblast specific factor 2 (fasciclin I-like) {Incyte PD: 1994715}	1994715	NM_006475
2.5	poly(A) polymerase {Incyte PD: 2231450}	2231450	AW205161
2.5	protein kinase C, theta {Incyte PD: 1844420}	1844420	AI570478
2.5	semenogelin I {Incyte PD: 4065623}	4065623	NM_003007
2.5	serine/threonine-protein kinase PRP4 homolog {Incyte PD: 604874}	604874	U48736
2.5	transcriptional adaptor 2 (ADA2, yeast, homolog)-like {Incyte PD: 2045628}	2045628	AW298572
2.5	TXK tyrosine kinase {Incyte PD: 1534482}	1534482	NM_003328
2.4	B melanoma antigen {Incyte PD: 2311955}	2311955	U19180
2.4	breast cancer anti-estrogen resistance 3 {Incyte PD: 998849}	998849	NM_003567
2.4	BTB and CNC homology 1, basic leucine zipper transcription factor 1 {Incyte PD: 1873492}	1873492	AI830904
2.4	cathepsin O {Incyte PD: 1689421}	1689421	AI816485
2.4	CDC23 (cell division cycle 23, yeast, homolog) {Incyte PD: 1534423}	1534423	AF053977
2.4	centromere protein A (17kD) {Incyte PD: 2444942}	2444942	NM_001809
2.4	CGG triplet repeat binding protein 1 {Incyte PD: 1428856}	1428856	AI608748
2.4	chemokine (C-X3-C) receptor 1 {Incyte PD: 2305611}	2305611	U20350
2.4	Cytokine-inducible kinase {Incyte PD: 1472377}	1472377	NM_004073
2.4	downregulated in ovarian cancer 1 {Incyte PD: 1685173}	1685173	U53445
2.4	epithelial V-like antigen 1 {Incyte PD: 1510349}	1510349	AI819274
2.4	EST {Incyte PD: 1541201}	1541201	AA157291
2.4	ESTs {Incyte PD: 1255873}	1255873	AA937212
2.4	ESTs {Incyte PD: 1284160}	1284160	AI758776
2.4	ESTs {Incyte PD: 1367516}	1367516	AA813998
2.4	ESTs {Incyte PD: 1382325}	1382325	AI761824
2.4	ESTs {Incyte PD: 1622323}	1622323	AW168774
2.4	ESTs {Incyte PD: 1842016}	1842016	AA505093
2.4	ESTs {Incyte PD: 2202515}	2202515	AI628916
2.4	ESTs {Incyte PD: 2246922}	2246922	AA706335
2.4	ESTs {Incyte PD: 2280833}	2280833	AI148361
2.4	ESTs {Incyte PD: 2293493}	2293493	AA993882
2.4	ESTs {Incyte PD: 2313003}	2313003	AA007604
2.4	ESTs {Incyte PD: 2753371}	2753371	AA993566

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.4	ESTs {Incyte PD: 2956964}	2956964	N62918
2.4	ESTs {Incyte PD: 3254319}	3254319	AI081832
2.4	ESTs {Incyte PD: 3881016}	3881016	AA868568
2.4	ESTs {Incyte PD: 4002274}	4002274	AA970119
2.4	ESTs {Incyte PD: 4055939}	4055939	AA847550
2.4	ESTs, Moderately similar to GLUTAMATE RECEPTOR, IONOTROPIC KAINATE 5 PRECURSOR [H.sapiens] {Incyte PD: 1889305}	1889305	AL134360
2.4	ESTs, Weakly similar to PHOSPHOLIPID HYDROPEROXIDE GLUTATHIONE PEROXIDASE [H.sapiens] {Incyte PD: 1664817}	1664817	N56736
2.4	eukaryotic translation initiation factor 4E binding protein 3 {Incyte PD: 1806736}	1806736	AA772089
2.4	gamma-aminobutyric acid (GABA) A receptor, alpha 5 {Incyte PD: 1291096}	1291096	NM_000810
2.4	Homo sapiens clone 23551 mRNA sequence {Incyte PD: 633326}	633326	AF007132
2.4	Homo sapiens clone 24627 mRNA sequence {Incyte PD: 2192092}	2192092	AF070618
2.4	Homo sapiens clone 24838 mRNA sequence {Incyte PD: 1507791}	1507791	AF131819
2.4	Homo sapiens clone IMAGE 21721 {Incyte PD: 3143449}	3143449	AI637917
2.4	Homo sapiens mRNA; cDNA DKFZp564C2163 (from clone DKFZp564C2163) {Incyte PD: 1752572}	1752572	AA189078
2.4	Human DNA sequence from clone 1183I21 on chromosome 20q12. Contains a novel gene and the first exon of a putative novel gene for a protein similar to predicted fly and worm proteins. Contains ESTs, STSs, GSSs and a putative CpG is {Incyte PD: 806803}	806803	AI564747
2.4	Incyte EST {Incyte PD: 1890987}	1890987	AK027199
2.4	Incyte EST {Incyte PD: 2025136}	2025136	N44825
2.4	Incyte EST {Incyte PD: 2348480}	2348480	AI674944
2.4	Incyte EST {Incyte PD: 3729702}	3729702	N66387
2.4	integrin, alpha 1 {Incyte PD: 537580}	537580	X68742
2.4	KIAA0758 protein {Incyte PD: 1965041}	1965041	AI093508
2.4	KIAA0774 protein {Incyte PD: 1833894}	1833894	AB018317
2.4	lamin B receptor {Incyte PD: 1803808}	1803808	L25931
2.4	major histocompatibility complex, class II, DM alpha {Incyte PD: 2059338}	2059338	X62744
2.4	mitogen-activated protein kinase kinase kinase kinase 5 {Incyte PD: 2293824}	2293824	AI683938
2.4	NADPH oxidase homolog-1mitogenic oxidase (pyridine nucleotide-dependent superoxide-generating) {Incyte PD: 2793437}	2793437	AI791894
2.4	nucleolar protein 4 {Incyte PD: 1596916}	1596916	AB015339
2.4	phosphodiesterase I/nucleotide pyrophosphatase 1 (homologous to mouse Ly-41 antigen) {Incyte PD: 1568123}	1568123	M57736
2.4	polymerase (DNA directed), epsilon 2 {Incyte PD: 4521835}	4521835	AF036899
2.4	Protease, serine, 7 (enterokinase) {Incyte PD: 1634539}	1634539	U09860
2.4	protein kinase, AMP-activated, alpha 2 catalytic subunit {Incyte PD: 2507648}	2507648	U06454
2.4	protein kinase, interferon-inducible double stranded RNA dependent {Incyte PD: 1510847}	1510847	M85294

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.4	protein phosphatase 1D magnesium-dependent, delta isoform {Incyte PD: 2748141}	2748141	NM_003620
2.4	RAB6 interacting, kinesin-like (rabkinesin6) {Incyte PD: 1975194}	1975194	NM_005733
2.4	regulator of G-protein signalling 13 {Incyte PD: 3696625}	3696625	AF030107
2.4	small inducible cytokine subfamily A (Cys-Cys), member 8 (monocyte chemotactic protein 2) {Incyte PD: 3734526}	3734526	AI590222
2.4	small inducible cytokine subfamily B (Cys-X-Cys), member 5 (epithelial-derived neutrophil-activating peptide 78) {Incyte PD: 4410949}	4410949	NM_002994
2.4	Small proline-rich protein SPRK [human, odontogenic keratocysts, mRNA Partial, 317 nt] {Incyte PD: 1599293}	1599293	AW238522
2.4	solute carrier family 16 (monocarboxylic acid transporters), member 1 {Incyte PD: 1981569}	1981569	AI565092
2.4	TGFB inducible early growth response {Incyte PD: 1806521}	1806521	AF050110
2.4	vascular endothelial growth factor {Incyte PD: 1861456}	1861456	AA285020
2.4	Vertebrate LIN7 homolog 1, Tax interaction protein 33 {Incyte PD: 1211072}	1211072	AA757170
2.4	X-ray repair complementing defective repair in Chinese hamster cells 4 {Incyte PD: 1681764}	1681764	AA909333
2.4	zinc finger protein 204 {Incyte PD: 1454640}	1454640	AW338359
2.3	acidic epididymal glycoprotein-like 1 {Incyte PD: 4065068}	4065068	X95237
2.3	Albumin {Incyte PD: 4087621}	4087621	AI207501
2.3	arginyltransferase 1 {Incyte PD: 1879587}	1879587	AF079099
2.3	cAMP responsive element binding protein 1 {Incyte PD: 2291535}	2291535	R68639
2.3	carboxypeptidase A3 (mast cell) {Incyte PD: 1707656}	1707656	M73720
2.3	caspase 3, apoptosis-related cysteine protease {Incyte PD: 3240708}	3240708	U13738
2.3	CD79B antigen (immunoglobulin-associated beta) {Incyte PD: 1646010}	1646010	M80461
2.3	CDC7 (cell division cycle 7, S. cerevisiae, homolog)-like 1 {Incyte PD: 2243929}	2243929	AF015592
2.3	centromere protein E (312kD) {Incyte PD: 3081067}	3081067	Z15005
2.3	cholinergic receptor, muscarinic 3 {Incyte PD: 2288609}	2288609	AI524284
2.3	chromosome 8 open reading frame 1 {Incyte PD: 1832530}	1832530	NM_004337
2.3	collagen, type IV, alpha 3 (Goodpasture antigen) {Incyte PD: 3535880}	3535880	M81379
2.3	deafness, autosomal dominant 5 {Incyte PD: 1491445}	1491445	AF075171
2.3	diaphanous (Drosophila, homolog) 2 {Incyte PD: 1448216}	1448216	Y15909
2.3	ectodermal dysplasia 1, anhidrotic {Incyte PD: 1445337}	1445337	AI015138
2.3	embryonic ectoderm development protein {Incyte PD: 2914968}	2914968	AI990456
2.3	EST {Incyte PD: 2371670}	2371670	AA868598
2.3	EST {Incyte PD: 3128093}	3128093	N25920
2.3	ESTs {Incyte PD: 1232365}	1232365	AI692432
2.3	ESTs {Incyte PD: 1300340}	1300340	AI161396
2.3	ESTs {Incyte PD: 1379395}	1379395	AA765113
2.3	ESTs {Incyte PD: 1417114}	1417114	AI018400
2.3	ESTs {Incyte PD: 1495766}	1495766	AA939266
2.3	ESTs {Incyte PD: 1781585}	1781585	AI379579
2.3	ESTs {Incyte PD: 1908884}	1908884	AI307360

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.3	ESTs {Incyte PD: 1995367}	1995367	AI027957
2.3	ESTs {Incyte PD: 2045472}	2045472	AI823412
2.3	ESTs {Incyte PD: 2054586}	2054586	AI638190
2.3	ESTs {Incyte PD: 2105026}	2105026	AA121270
2.3	ESTs {Incyte PD: 2265209}	2265209	AA058640
2.3	ESTs {Incyte PD: 2313368}	2313368	AA287489
2.3	ESTs {Incyte PD: 2606738}	2606738	AI018775
2.3	ESTs {Incyte PD: 2858866}	2858866	AA281719
2.3	ESTs {Incyte PD: 3149856}	3149856	AI498125
2.3	ESTs {Incyte PD: 4106720}	4106720	AA782081
2.3	ESTs {Incyte PD: 544213}	544213	AW024492
2.3	ESTs {Incyte PD: 544601}	544601	AW021108
2.3	ESTs, Highly similar to map kinase phosphatase-like protein MK-STYX [H.sapiens] {Incyte PD: 1810903}	1810903	AW055105
2.3	ESTs, Moderately similar to ORF derived from protease and integrase coding regions [H.sapiens] {Incyte PD: 2082211}	2082211	AA410508
2.3	ESTs, Weakly similar to GTPase-activating protein II [H.sapiens] {Incyte PD: 1304879}	1304879	AA766027
2.3	ESTs, Weakly similar to phosphoinositide 3-kinase [H.sapiens] {Incyte PD: 1415307}	1415307	AI823970
2.3	exonuclease 1 {Incyte PD: 4385292}	4385292	AF042282
2.3	FGFR1 oncogene partner {Incyte PD: 1919724}	1919724	NM_007045
2.3	FSH primary response (LRPR1, rat) homolog 1 {Incyte PD: 2542561}	2542561	NM_006733
2.3	gamma-aminobutyric acid (GABA) A receptor, pi {Incyte PD: 1824443}	1824443	U95367
2.3	golgi SNAP receptor complex member 1 {Incyte PD: 1427756}	1427756	AW029303
2.3	hematopoietically expressed homeobox {Incyte PD: 1680580}	1680580	X67235
2.3	Histatin 3 {Incyte PD: 1323722}	1323722	AA376718
2.3	Histone acetyltransferase 1 {Incyte PD: 3485789}	3485789	AF030424
2.3	homeo box A4 {Incyte PD: 1703133}	1703133	NM_002141
2.3	Homo sapiens clone 24461 mRNA sequence {Incyte PD: 1807613}	1807613	AF070577
2.3	Homo sapiens mRNA from chromosome 5q21-22, clone:357Ex {Incyte PD: 1920250}	1920250	AB002448
2.3	Homo sapiens mRNA; cDNA DKFZp564F013 (from clone DKFZp564F013) {Incyte PD: 3323473}	3323473	AL042439
2.3	Homo sapiens platelet activating receptor homolog (H963) mRNA, complete cds {Incyte PD: 3879095}	3879095	AF002986
2.3	Human clone 23560 mRNA sequence {Incyte PD: 4769031}	4769031	AW297388
2.3	Human clone 23948 mRNA sequence {Incyte PD: 3123244}	3123244	U79293
2.3	Human clone CE29 7.2 (CAC)n/(GTG)n repeat-containing mRNA {Incyte PD: 3712928}	3712928	AA765005
2.3	hyaluronan-mediated motility receptor (RHAMM) {Incyte PD: 3622417}	3622417	U29343
2.3	IGF-II mRNA-binding protein 3 {Incyte PD: 1420380}	1420380	NM_006547
2.3	Incyte EST {Incyte PD: 3001809}	3001809	BI009708
2.3	Incyte EST {Incyte PD: 3143102}	3143102	AI004747
2.3	Incyte EST {Incyte PD: 396954}	396954	
2.3	Incyte EST {Incyte PD: 862356}	862356	AI914410

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.3	interferon stimulated gene (20kD) {Incyte PD: 2238363}	2238363	X89773
2.3	keratocan {Incyte PD: 2804463}	2804463	NM_007035
2.3	KIAA0057 gene product; TRAM-like protein {Incyte PD: 1961828}	1961828	D31762
2.3	KIAA0445 gene product {Incyte PD: 2119925}	2119925	AB007914
2.3	Kinesin-like 4 {Incyte PD: 2760114}	2760114	AB017430
2.3	leptin receptor {Incyte PD: 1849302}	1849302	U52914
2.3	leukotriene A4 hydrolase {Incyte PD: 1988019}	1988019	AW236605
2.3	lipoma HMGIC fusion partner-like 2 {Incyte PD: 1964603}	1964603	D86961
2.3	low density lipoprotein receptor (familial hypercholesterolemia) {Incyte PD: 1986809}	1986809	AW006976
2.3	malic enzyme 1, NADP(+)-dependent, cytosolic {Incyte PD: 972390}	972390	AL049699
2.3	matrix metalloproteinase 12 (macrophage elastase) {Incyte PD: 1563275}	1563275	U78045
2.3	methionine adenosyltransferase II, alpha {Incyte PD: 1619980}	1619980	AW364505
2.3	microphthalmia-associated transcription factor {Incyte PD: 1749130}	1749130	N33162
2.3	NCK adaptor protein 1 {Incyte PD: 1833038}	1833038	AI754384
2.3	postmeiotic segregation increased (S. cerevisiae) 1 {Incyte PD: 520140}	520140	U13695
2.3	prominin (mouse)-like 1 {Incyte PD: 2070568}	2070568	AF027208
2.3	putative brain nuclearly-targeted protein {Incyte PD: 2742315}	2742315	W92591
2.3	regulatory factor X, 5 (influences HLA class II expression) {Incyte PD: 1720973}	1720973	AI127781
2.3	retinoblastoma-binding protein 6 {Incyte PD: 1879135}	1879135	AI184316
2.3	serine/threonine kinase 17a (apoptosis-inducing) {Incyte PD: 2416415}	2416415	NM_004760
2.3	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase; GM3 synthase) {Incyte PD: 603416}	603416	AI423978
2.3	small inducible cytokine subfamily B (Cys-X-Cys), member 10 {Incyte PD: 1656473}	1656473	NM_001565
2.3	Thyroid stimulating hormone, beta {Incyte PD: 1758520}	1758520	S70587
2.3	transcription factor 21 {Incyte PD: 1851913}	1851913	AI262045
2.3	transmembrane trafficking protein {Incyte PD: 11556}	11556	W37571
2.3	ubiquitin activating enzyme E1-like protein {Incyte PD: 2396858}	2396858	NM_006395
2.3	v-myb avian myeloblastosis viral oncogene homolog-like 1 {Incyte PD: 1879041}	1879041	AA761901
2.3	YY1 transcription factor {Incyte PD: 931833}	931833	AI471525
2.2	A kinase (PRKA) anchor protein 9 {Incyte PD: 3868809}	3868809	NM_005751
2.2	aminopeptidase puromycin sensitive {Incyte PD: 1835053}	1835053	AA804261
2.2	antigenic determinant of recA protein (mouse) homolog {Incyte PD: 4514031}	4514031	AW001823
2.2	apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 {Incyte PD: 1634063}	1634063	AB009426
2.2	apoptosis inhibitor 4 (survivin) {Incyte PD: 1645766}	1645766	U75285
2.2	BMX non-receptor tyrosine kinase {Incyte PD: 1655995}	1655995	AF045459
2.2	calcium channel, voltage-dependent, L type, alpha 1C subunit {Incyte PD: 3448231}	3448231	Z34817

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.2	chemokine (C-C motif) receptor 5 {Incyte PD: 4240009}	4240009	U54994
2.2	chromosome condensation 1-like {Incyte PD: 2359841}	2359841	NM_001268
2.2	cullin 5 {Incyte PD: 3720083}	3720083	X81882
2.2	cyclin G2 {Incyte PD: 1922164}	1922164	AI271688
2.2	cytochrome P450, subfamily IIE (ethanol-inducible) {Incyte PD: 294379}	294379	J02843
2.2	delta-like 1 (mouse) homolog {Incyte PD: 2272122}	2272122	AW274267
2.2	development and differentiation enhancing factor 2 {Incyte PD: 1923070}	1923070	NM_003887
2.2	DKFZP564M112 protein {Incyte PD: 521943}	521943	AI346927
2.2	DKFZP586B0923 protein {Incyte PD: 1336305}	1336305	AA044848
2.2	early endosome antigen 1, 162kD {Incyte PD: 4227930}	4227930	NM_003566
2.2	EST {Incyte PD: 3220149}	3220149	H03260
2.2	ESTs {Incyte PD: 1267613}	1267613	N63894
2.2	ESTs {Incyte PD: 1348181}	1348181	AI042261
2.2	ESTs {Incyte PD: 1375813}	1375813	AI049723
2.2	ESTs {Incyte PD: 1397926}	1397926	N35555
2.2	ESTs {Incyte PD: 1478813}	1478813	AI672174
2.2	ESTs {Incyte PD: 1601930}	1601930	AI801963
2.2	ESTs {Incyte PD: 1679304}	1679304	AI810024
2.2	ESTs {Incyte PD: 1732437}	1732437	AA824391
2.2	ESTs {Incyte PD: 1844052}	1844052	AA574098
2.2	ESTs {Incyte PD: 1880335}	1880335	AA496402
2.2	ESTs {Incyte PD: 1919233}	1919233	AA633977
2.2	ESTs {Incyte PD: 1923117}	1923117	AI684590
2.2	ESTs {Incyte PD: 1985701}	1985701	N98410
2.2	ESTs {Incyte PD: 1998860}	1998860	AI445726
2.2	ESTs {Incyte PD: 2111230}	2111230	AI744715
2.2	ESTs {Incyte PD: 2255215}	2255215	AI540220
2.2	ESTs {Incyte PD: 2289901}	2289901	AA992403
2.2	ESTs {Incyte PD: 2319421}	2319421	AI418933
2.2	ESTs {Incyte PD: 2327380}	2327380	AA040154
2.2	ESTs {Incyte PD: 2396385}	2396385	AI026691
2.2	ESTs {Incyte PD: 2398485}	2398485	AI276944
2.2	ESTs {Incyte PD: 2652916}	2652916	AI499800
2.2	ESTs {Incyte PD: 2674167}	2674167	N32192
2.2	ESTs {Incyte PD: 3144229}	3144229	AA721277
2.2	ESTs {Incyte PD: 3144809}	3144809	AA912872
2.2	ESTs {Incyte PD: 3561334}	3561334	T93186
2.2	ESTs {Incyte PD: 3571330}	3571330	N46420
2.2	ESTs {Incyte PD: 3645701}	3645701	AA731863
2.2	ESTs {Incyte PD: 4243663}	4243663	Z39995
2.2	ESTs {Incyte PD: 532490}	532490	AI809937
2.2	ESTs {Incyte PD: 639847}	639847	AA156906
2.2	ESTs {Incyte PD: 812412}	812412	AI274567
2.2	ESTs {Incyte PD: 998392}	998392	AA418721
2.2	ESTs, Moderately similar to hypothetical protein [H.sapiens] {Incyte PD: 1540157}	1540157	AI186031
2.2	ESTs, Weakly similar to fatty acid omega-hydroxylase	3441613	AW275990

Gene Expression Ratio	GeneName	CloneID	Accession Number
.	[H.sapiens] {Incyte PD: 3441613}		
2.2	ESTs, Weakly similar to gc-rich sequence dna-binding factor [H.sapiens] {Incyte PD: 1358710}	1358710	AI961320
2.2	ESTs, Weakly similar to KIAA0594 protein [H.sapiens] {Incyte PD: 1309504}	1309504	AW183697
2.2	fer (fps/fes related) tyrosine kinase (phosphoprotein NCP94) {Incyte PD: 4606375}	4606375	J03358
2.2	fibrinogen-like 1 {Incyte PD: 4284270}	4284270	NM_004467
2.2	Fibulin 4 {Incyte PD: 1967983}	1967983	AI394645
2.2	forkhead box C1 {Incyte PD: 1622613}	1622613	AW195359
2.2	four and a half LIM domains 2 {Incyte PD: 2131914}	2131914	AA515766
2.2	FSHD region gene 1 {Incyte PD: 3476010}	3476010	AI125735
2.2	G antigen 2 {Incyte PD: 1979733}	1979733	U19143
2.2	gonadotropin-releasing hormone 1 (leutinizing-releasing hormone) {Incyte PD: 1830640}	1830640	X01059
2.2′	GSK-3 binding protein FRAT2 {Incyte PD: 3871545}	3871545	AA442314
2.2	H.sapiens mRNA for mitotic kinesin-like protein-1 {Incyte PD: 2640427}	2640427	H63163
2.2	H2B histone family, member R {Incyte PD: 4997978}	4997978	AL138326
2.2	Homo sapiens clone 23849 mRNA sequence {Incyte PD: 2395017}	2395017	AA406448
2.2	Homo sapiens clone 23926 mRNA sequence {Incyte PD: 1457711}	1457711	AA765137
2.2	Homo sapiens clone 24630 mRNA sequence {Incyte PD: 1476214}	1476214	AF052174
2.2	Homo sapiens clone 643 unknown mRNA, complete sequence {Incyte PD: 1451876}	1451876	AF091087
2.2	Homo sapiens mRNA for lipoyltransferase, complete cds {Incyte PD: 1800104}	1800104	AB017567
2.2	Homo sapiens mRNA; cDNA DKFZp564C2163 (from clone DKFZp564C2163) {Incyte PD: 2276037}	2276037	AI860320
2.2	Homo sapiens pre-mRNA splicing SR protein rA4 mRNA, partial cds {Incyte PD: 2601777}	2601777	AI937268
2.2	Human Ig J chain gene {Incyte PD: 1001933}	1001933	AW172754
2.2	Incyte EST {Incyte PD: 400429}	400429	NM_000306
2.2	indoleamine-pyrrole 2,3 dioxygenase {Incyte PD: 1749102}	1749102	NM_002164
2.2	inositol(myo)-1(or 4)-monophosphatase 1 {Incyte PD: 1403168}	1403168	Y11360
2.2	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor) {Incyte PD: 2803366}	2803366	NM_000885
2.2	interleukin 1, alpha {Incyte PD: 557538}	557538	M28983
2.2	KIAA0029 protein {Incyte PD: 1290568}	1290568	AA824294
2.2	KIAA0250 gene product {Incyte PD: 518495}	518495	D87437
2.2	KIAA0535 gene product {Incyte PD: 1311071}	1311071	AB011107
2.2	KIAA0678 protein {Incyte PD: 1692516}	1692516	AA669086
2.2	KIAA0751 gene product {Incyte PD: 1760318}	1760318	AA476826
2.2	KIAA0753 gene product {Incyte PD: 2498968}	2498968	AL080108
2.2	KIAA0768 protein {Incyte PD: 1739640}	1739640	AB018311
2.2	KIAA1025 protein {Incyte PD: 2125530}	2125530	AI250775
2.2	Kreisler (mouse) maf-related leucine zipper homolog {Incyte PD: 2600080}	2600080	NM_005461
2.2	LIM and senescent cell antigen-like domains 1 {Incyte PD:	631262	AI318413

Gene Expression Ratio	GeneName	CloneID	Accession Number
	631262}		
2.2	loss of heterozygosity, 11, chromosomal region 2, gene A {Incyte PD: 2760753}	2760753	AW104055
2.2	lumican {Incyte PD: 1228124}	1228124	AL036211
2.2	membrane-spanning 4-domains, subfamily A, member 1 (Fc fragment of IgE, high affinity I, receptor for; beta polypeptide) {Incyte PD: 3742039}	3742039	M89796
2.2	minichromosome maintenance deficient (S. cerevisiae) 3 {Incyte PD: 1610083}	1610083	NM_002388
2.2	myosin regulatory light chain 2, smooth muscle isoform {Incyte PD: 3808966}	3808966	AI218457
2.2	myotubularin related protein 7 {Incyte PD: 3837624}	3837624	AF073482
2.2	PDZ domain protein (Drosophila inaD-like) {Incyte PD: 2359622}	2359622	AJ001306
2.2	phosphate regulating gene with homologies to endopeptidases on the X chromosome (hypophosphatemia, vitamin D resistant rickets) {Incyte PD: 530645}	530645	Y10196
2.2	phosphodiesterase 3A, cGMP-inhibited {Incyte PD: 1512943}	1512943	U36798
2.2	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase {Incyte PD: 1929290}	1929290	AI144165
2.2	platelet-derived growth factor receptor, alpha polypeptide {Incyte PD: 1705274}	1705274	AI805230
2.2	potassium inwardly-rectifying channel, subfamily J, member 1 {Incyte PD: 957604}	957604	U03884
2.2	regulator of Gz-selective protein signaling {Incyte PD: 4711030}	4711030	NM_003702
2.2	SCO (cytochrome oxidase deficient, yeast) homolog 1 {Incyte PD: 644927}	644927	AI332708
2.2	selectin E (endothelial adhesion molecule 1) {Incyte PD: 3357888}	3357888	M30640
2.2	sin3-associated polypeptide, 18kD {Incyte PD: 2293401}	2293401	AI590320
2.2	syntaxin 7 {Incyte PD: 1984908}	1984908	AA830963
2.2	transmembrane protein 1 {Incyte PD: 2285180}	2285180	U61500
2.2	tropomyosin 1 (alpha) {Incyte PD: 2291135}	2291135	N76371
2.2	uroplakin 1B {Incyte PD: 2932992}	2932992	AI982899
2.2	vacuolar protein sorting 45B (yeast homolog) {Incyte PD: 1396925}	1396925	AA765898
2.2	vav 1 oncogene {Incyte PD: 1684960}	1684960	AI886795
2.2	v-myb avian myeloblastosis viral oncogene homolog {Incyte PD: 2555590}	2555590	U22376
2.2	zinc finger protein homologous to Zfp161 in mouse {Incyte PD: 2487368}	2487368	D89859
2.1	A kinase (PRKA) anchor protein 9 {Incyte PD: 1996806}	1996806	NM_005751
2.1	absent in melanoma 1 {Incyte PD: 2075425}	2075425	U83115
2.1	acid sphingomyelinase-like phosphodiesterase {Incyte PD: 1695477}	1695477	Y08136
2.1	acylphosphatase 1, erythrocyte (common) type {Incyte PD: 1995749}	1995749	AL039701
2.1	alkaline phosphatase, placental (Regan isozyme) {Incyte PD: 4401302}	4401302	X53279
2.1	anaphase-promoting complex 10 {Incyte PD: 4216962}	4216962	AA262889

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.1	Arginine-rich protein {Incyte PD: 1618455}	1618455	AI092884
2.1	BCL2-related protein A1 {Incyte PD: 2555673}	2555673	U29680
2.1	betaine-homocysteine methyltransferase {Incyte PD: 139449}	139449	U50929
2.1	brefeldin A-sensitive, peripheral Golgi protein {Incyte PD: 1468607}	1468607	Z34975
2.1	bromodomain, testis-specific {Incyte PD: 3145490}	3145490	AA884041
2.1	butyrylcholinesterase {Incyte PD: 1599272}	1599272	NM_000055
2.1	carbonic anhydrase VIII {Incyte PD: 4714232}	4714232	AI580352
2.1	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1 {Incyte PD: 1756219}	1756219	AI041143
2.1	CD27-binding (Siva) protein {Incyte PD: 2356635}	2356635	AI267883
2.1	CD3D antigen, delta polypeptide (TiT3 complex) {Incyte PD: 3297914}	3297914	AA310902
2.1	ceruloplasmin (ferroxidase) {Incyte PD: 1982416}	1982416	NM_000096
2.1	chromosome 11 open reading frame 8 {Incyte PD: 4117578}	4117578	AI951765
2.1	chromosome X open reading frame 5 {Incyte PD: 2080379}	2080379	AA173595
2.1	claudin 7 {Incyte PD: 2060261}	2060261	AI279608
2.1	C-terminal binding protein 2 {Incyte PD: 1909609}	1909609	AF016507
2.1	cyclin G1 {Incyte PD: 1684890}	1684890	U53328
2.1	cyclin-dependent kinase 8 {Incyte PD: 2756066}	2756066	AI657156
2.1	DEK gene {Incyte PD: 1980248}	1980248	X64229
2.1	deleted in azoospermia-like {Incyte PD: 4919920}	4919920	U66078
2.1	disabled (Drosophila) homolog 2 (mitogen-responsive phosphoprotein) {Incyte PD: 1976279}	1976279	NM_001343
2.1	DKFZP434D174 protein {Incyte PD: 1496005}	1496005	AI569602
2.1	DNA polymerase delta, subunit 3 {Incyte PD: 701596}	701596	D26018
2.1	DNA segment on chromosome X and Y (unique) 155 expressed sequence {Incyte PD: 1812440}	1812440	N42070
2.1	Down syndrome critical region protein A {Incyte PD: 1494184}	1494184	NM_006052
2.1	down-regulated in adenoma {Incyte PD: 1842009}	1842009	NM_000111
2.1	ESTs {Incyte PD: 1290212}	1290212	AW294859
2.1	ESTs {Incyte PD: 1376651}	1376651	AA004211
2.1	ESTs {Incyte PD: 1400087}	1400087	AA780250
2.1	ESTs {Incyte PD: 1431471}	1431471	AW293495
2.1	ESTs {Incyte PD: 1449467}	1449467	W35325
2.1	ESTs {Incyte PD: 1506962}	1506962	AI023426
2.1	ESTs {Incyte PD: 1615819}	1615819	AI917371
2.1	ESTs {Incyte PD: 1680730}	1680730	AA872191
2.1	ESTs {Incyte PD: 1803882}	1803882	N79249
2.1	ESTs {Incyte PD: 1824332}	1824332	AA993406
2.1	ESTs {Incyte PD: 1843971}	1843971	AI307111
2.1	ESTs {Incyte PD: 1908734}	1908734	AI187136
2.1	ESTs {Incyte PD: 1923638}	1923638	AA749317
2.1	ESTs {Incyte PD: 1996180}	1996180	AA279943
2.1	ESTs {Incyte PD: 2019032}	2019032	AI243383
2.1	ESTs {Incyte PD: 2073590}	2073590	AA976388
2.1	ESTs {Incyte PD: 2105590}	2105590	AI672106
2.1	ESTs {Incyte PD: 2121661}	2121661	N66734
2.1	ESTs {Incyte PD: 2176387}	2176387	N33144

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.1	ESTs {Incyte PD: 2183334}	2183334	AW293233
2.1	ESTs {Incyte PD: 2279230}	2279230	AI581085
2.1	ESTs {Incyte PD: 2285983}	2285983	AW337854
2.1	ESTs {Incyte PD: 2343492}	2343492	AA976583
2.1	ESTs {Incyte PD: 2369022}	2369022	AI563922
2.1	ESTs {Incyte PD: 2397579}	2397579	AI187051
2.1	ESTs {Incyte PD: 2462127}	2462127	AI859289
2.1	ESTs {Incyte PD: 2584766}	2584766	AI688586
2.1	ESTs {Incyte PD: 2589143}	2589143	AA846803
2.1	ESTs {Incyte PD: 2591193}	2591193	AI087339
2.1	ESTs {Incyte PD: 2676494}	2676494	AA453418
2.1	ESTs {Incyte PD: 2749990}	2749990	AA598853
2.1	ESTs {Incyte PD: 2880627}	2880627	AA290901
2.1	ESTs {Incyte PD: 2906194}	2906194	N26569
2.1	ESTs {Incyte PD: 3045817}	3045817	AA812741
2.1	ESTs {Incyte PD: 3427294}	3427294	AA400519
2.1	ESTs {Incyte PD: 3510725}	3510725	H07955
2.1	ESTs {Incyte PD: 3728524}	3728524	AA936753
2.1	ESTs {Incyte PD: 3880684}	3880684	AA700590
2.1	ESTs {Incyte PD: 495729}	495729	W94993
2.1	ESTs {Incyte PD: 539603}	539603	AI367621
2.1	ESTs {Incyte PD: 559786}	559786	AI821999
2.1	ESTs {Incyte PD: 695016}	695016	AI086855
2.1	ESTs {Incyte PD: 931235}	931235	AA479746
2.1	ESTs {Incyte PD: 938904}	938904	AI142885
2.1	ESTs {Incyte PD: 961630}	961630	AW173154
2.1	ESTs {Incyte PD: 997494}	997494	AI355014
2.1	ESTs, Highly similar to unknown function [H.sapiens] {Incyte PD: 2905005}	2905005	AA156947
2.1	ESTs, Moderately similar to MAGUK P55 SUBFAMILY MEMBER 2 [H.sapiens] {Incyte PD: 2171614}	2171614	AI147946
2.1	ESTs, Weakly similar to /prediction {Incyte PD: 2046439}	2046439	AA608632
2.1	ESTs, Weakly similar to CGI-41 protein [H.sapiens] {Incyte PD: 2600028}	2600028	AI310001
2.1	ESTs, Weakly similar to Closely related to Arabidopsis thaliana gene T9J22.7 [C.elegans] {Incyte PD: 2058620}	2058620	AA725579
2.1	ESTs, Weakly similar to ORF YKR079c [S.cerevisiae] {Incyte PD: 2671743}	2671743	AI807344
2.1	ESTs, Weakly similar to putative [C.elegans] {Incyte PD: 3333715}	3333715	AI308071
2.1	eukaryotic translation initiation factor 4E {Incyte PD: 1901069}	1901069	M15353
2.1	extracellular matrix protein 2, female organ and adipocyte specific {Incyte PD: 2886607}	2886607	AB011792
2.1	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide {Incyte PD: 2762987}	2762987	J03605
2.1	fibrinogen, A alpha polypeptide {Incyte PD: 1511658}	1511658	M64982
2.1	G protein-coupled receptor 65 {Incyte PD: 635562}	635562	AI017452
2.1	general transcription factor IIE, polypeptide 1 (alpha subunit, 56kD) {Incyte PD: 3203823}	3203823	AL135122
2.1	glutamate receptor, metabotropic 1 {Incyte PD: 3335010}	3335010	NM_000838

Gene Expression Ratio	GeneName	CloneID	Accession Number
2.1	golgi autoantigen, golgin subfamily a, 5 {Incyte PD: 2068966}	2068966	AF085199
2.1	Golgi vesicular membrane trafficking protein p18 {Incyte PD: 1429267}	1429267	AW327720
2.1	granzyme K (serine protease, granzyme 3; tryptase II) {Incyte PD: 1822519}	1822519	AA947915
2.1	GTP-binding protein ragB {Incyte PD: 2601422}	2601422	AA234339
2.1	heterogeneous nuclear ribonucleoprotein A/B {Incyte PD: 2194749}	2194749	AI277400
2.1	histidine ammonia-lyase {Incyte PD: 3380034}	3380034	D16626
2.1	holocytochrome c synthase (cytochrome c heme-lyase) {Incyte PD: 1522477}	1522477	NM_005333
2.1	Homo sapiens chromosome 19, cosmid R26894 {Incyte PD: 2875486}	2875486	AA534193
2.1	Homo sapiens clone 24707 mRNA sequence {Incyte PD: 645397}	645397	AF055007
2.1	Homo sapiens clone 24820 mRNA sequence {Incyte PD: 4032372}	4032372	AF070547
2.1	Homo sapiens clone 25242 mRNA sequence {Incyte PD: 1879888}	1879888	N31370
2.1	Homo sapiens clones 24714 and 24715 mRNA sequence {Incyte PD: 2599229}	2599229	R56641
2.1	Homo sapiens Dickkopf-1 (hdkk-1) gene {Incyte PD: 5047895}	5047895	AB020315
2.1	Homo sapiens mRNA for G3a protein (G3a gene, located in the class III region of the major histocompatibility complex) {Incyte PD: 1958226}	1958226	AI858956
2.1	Homo sapiens mRNA in the region near the btk gene involved in a-gamma-globulinemia {Incyte PD: 1910380}	1910380	AA398519
2.1	Homo sapiens mRNA; cDNA DKFZp564D0462 (from clone DKFZp564D0462) {Incyte PD: 1803721}	1803721	AL080079
2.1	Homo sapiens PAC clone DJ1121E10 from 7q21.1-q2 {Incyte PD: 1496753}	1496753	AC005053
2.1	Human DNA sequence from clone 596C15 on chromosome Xq23. Contains the GUCY2F gene for guanylate cyclase 2F, retinal (EC 4.6.1.2, RETGC-2, Rod Outer Segment Membrane Guanylate Cyclase 2, ROS-GC2, GC-F) and a potentially alternative {Incyte PD: 2239005}	2239005	AL031387
2.1	hydroxyprostaglandin dehydrogenase 15-(NAD) {Incyte PD: 1578941}	1578941	NM_000860
2.1	Incyte EST {Incyte PD: 1311471}	1311471	AA342271
2.1	Incyte EST {Incyte PD: 1998288}	1998288	AI342337
2.1	Incyte EST {Incyte PD: 2045755}	2045755	AA056610
2.1	Incyte EST {Incyte PD: 2054426}	2054426	N57273
2.1	Incyte EST {Incyte PD: 2073339}	2073339	AL536250
2.1	Incyte EST {Incyte PD: 2612856}	2612856	BG822718
2.1	Incyte EST {Incyte PD: 3364304}	3364304	
2.1	Incyte EST {Incyte PD: 3736683}	3736683	AI923137
2.1	Incyte EST {Incyte PD: 4630674}	4630674	NM_006566
2.1	interferon (alpha, beta and omega) receptor 2 {Incyte PD: 1683165}	1683165	L41942
2.1	interleukin 4 {Incyte PD: 4347370}	4347370	NM_000589
2.1	isocitrate dehydrogenase 1 (NADP+), soluble {Incyte PD:	1981403	AI284981

Gene Expression Ratio	GeneName	CloneID	Accession Number
	1981403}		
2.1	KIAA0062 protein {Incyte PD: 3138128}	3138128	D31887
2.1	KIAA0215 gene product {Incyte PD: 3172946}	3172946	D86969
2.1	KIAA0241 protein {Incyte PD: 2754116}	2754116	D87682
2.1	KIAA0286 protein {Incyte PD: 2689016}	2689016	AB006624
2.1	KIAA0699 protein {Incyte PD: 522905}	522905	AI698095
2.1	KIAA0771 protein {Incyte PD: 2193506}	2193506	AB018314
2.1	KIAA0808 gene product {Incyte PD: 3230705}	3230705	AB018351
2.1	Kruppel-like factor 4 (gut) {Incyte PD: 1962235}	1962235	AA573434
2.1	LIM domain only 7 {Incyte PD: 1832584}	1832584	AI148755
2.1	low density lipoprotein receptor-related protein 6 {Incyte PD: 2297714}	2297714	N71497
2.1	mutS (E. coli) homolog 5 {Incyte PD: 1673638}	1673638	AF048991
2.1	myosin IE {Incyte PD: 1215472}	1215472	AW276523
2.1	myosin-binding protein C, cardiac {Incyte PD: 3601843}	3601843	AI650915
2.1	nel (chicken)-like 2 {Incyte PD: 2285502}	2285502	NM_006159
2.1	Novel human gene mapping to chomosome 13 {Incyte PD: 1297988}	1297988	U57962
2.1	novel putative protein similar to YIL091C yeast hypothetical 84 kD protein from SGA1-KTR7 {Incyte PD: 2844869}	2844869	AL022398
2.1	nuclear receptor subfamily 3, group C, member 2 {Incyte PD: 1678071}	1678071	NM_000901
2.1	nudix (nucleoside diphosphate linked moiety X)-type motif 3 {Incyte PD: 2966564}	2966564	AI589855
2.1	osmosis responsive factor {Incyte PD: 2078614}	2078614	AF023244
2.1	patched related protein translocated in renal cancer {Incyte PD: 2398148}	2398148	AI805452
2.1	phospholipase D1, phophatidylcholine-specific {Incyte PD: 1330492}	1330492	NM_002662
2.1	phosphoribosyl pyrophosphate synthetase 2 {Incyte PD: 2054641}	2054641	N52940
2.1	plastin 1 (I isoform) {Incyte PD: 609115}	609115	NM_002670
2.1	plexin C1 {Incyte PD: 1554732}	1554732	AF035307
2.1	prostaglandin E receptor 2 (subtype EP2), 53kD {Incyte PD: 1500365}	1500365	U19487
2.1	prostate differentiation factor {Incyte PD: 2042056}	2042056	AB000584
2.1	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional protease 2) {Incyte PD: 2018222}	2018222	AI923532
2.1	protein kinase C, mu {Incyte PD: 957345}	957345	X75756
2.1	RAB28, member RAS oncogene family {Incyte PD: 1457948}	1457948	NM_004249
2.1	RAE1 (RNA export 1, S.pombe) homolog {Incyte PD: 588157}	588157	AI857816
2.1	ras homolog gene family, member E {Incyte PD: 1794550}	1794550	W03441
2.1	rat regenerating islet-derived-like, human homolog (pancreatic stone protein-like, pancreatic thread protein-like) {Incyte PD: 2068983}	2068983	D56495
2.1	ret finger protein {Incyte PD: 2708651}	2708651	AI798574
2.1	ring finger protein 3 {Incyte PD: 2860918}	2860918	AA403225
2.1	small inducible cytokine subfamily A (Cys-Cys), member 13 {Incyte PD: 1977527}	1977527	U59808
2.1	small nuclear RNA activating complex, polypeptide 3, 50kD	2743918	AA829743

Gene Expression Ratio	GeneName	CloneID	Accession Number
	{Incyte PD: 2743918}		
2.1	snail 1 (drosophila homolog), zinc finger protein {Incyte PD: 2398618}	2398618	AI218759
2.1	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2 {Incyte PD: 1684954}	1684954	AI801003
2.1	solute carrier family 22 (organic cation transporter), member 2 {Incyte PD: 4337804}	4337804	NM_003058
2.1	sorbitol dehydrogenase {Incyte PD: 1856587}	1856587	AI458045
2.1	Sp3 transcription factor {Incyte PD: 1642252}	1642252	X68560
2.1	statherin {Incyte PD: 1825492}	1825492	M18371
2.1	survival of motor neuron protein interacting protein 1 {Incyte PD: 2967782}	2967782	AF027150
2.1	testis specific leucine rich repeat protein {Incyte PD: 1929279}	1929279	U60666
2.1	thyroid autoantigen 70kD (Ku antigen) {Incyte PD: 1995895}	1995895	AA576761
2.1	thyroxin-binding globulin {Incyte PD: 4795635}	4795635	Z83850
2.1	toll-like receptor 1 {Incyte PD: 1241477}	1241477	AL050262
2.1	transcription factor EC {Incyte PD: 2470390}	2470390	D43945
2.1	trinucleotide repeat containing 9 {Incyte PD: 863708}	863708	U80736
2.1	tumor necrosis factor, alpha-induced protein 6 {Incyte PD: 3142364}	3142364	M31165
2.1	tumor protein p53-binding protein {Incyte PD: 659473}	659473	NM_005802
2.1	UDP-N-acteylglucosamine pyrophosphorylase 1; Sperm associated antigen 2 {Incyte PD: 1997038}	1997038	AA448531
2.1	uridine monophosphate kinase {Incyte PD: 1959569}	1959569	AA468735
2.1	vaccinia related kinase 1 {Incyte PD: 3116351}	3116351	AA312869
2.1	Vanin 1 {Incyte PD: 3843507}	3843507	AJ132099
2.1	vesicle docking protein p115 {Incyte PD: 1714811}	1714811	NM_003715
2.1	Werner syndrome {Incyte PD: 1634538}	1634538	NM_000553
2.1	zinc finger protein 217 {Incyte PD: 1926657}	1926657	AW085283
2.1	zinc finger protein 23 (KOX 16) {Incyte PD: 1617274}	1617274	AI146709
2	3-oxoacid CoA transferase {Incyte PD: 1685342}	1685342	U62961
2	aminolevulinate, delta-, synthase 1 {Incyte PD: 943569}	943569	AA707391
2	ankyrin-like with transmembrane domains 1 {Incyte PD: 3529505}	3529505	Y10601
2	APG5 (autophagy 5, S. cerevisiae)-like {Incyte PD: 2291611}	2291611	AL022067
2	apoptosis inhibitor 4 (survivin) {Incyte PD: 2504586}	2504586	AW247335
2	ataxia telangiectasia and Rad3 related {Incyte PD: 632440}	632440	U49844
2	ATPase, Cu++ transporting, alpha polypeptide (Menkes syndrome) {Incyte PD: 2929674}	2929674	NM_000052
2	ATP-binding cassette, sub-family E (OABP), member 1 {Incyte PD: 1674405}	1674405	NM_002940
2	basic leucine zipper nuclear factor 1 (JEM-1) {Incyte PD: 2459178}	2459178	AI887216
2	BCL2-associated athanogene 5 {Incyte PD: 3002423}	3002423	NM_004873
2	betaine-homocysteine methyltransferase {Incyte PD: 763477}	763477	U50929
2	brain-specific angiogenesis inhibitor 3 {Incyte PD: 3600367}	3600367	AB005299
2	breast cell glutaminase {Incyte PD: 1781632}	1781632	AF038170
2	Bruton agammaglobulinemia tyrosine kinase {Incyte PD: 2204867}	2204867	U78027
2	cadherin 1, E-cadherin (epithelial) {Incyte PD: 1208946}	1208946	N90163

Gene Expression Ratio	GeneName	CloneID	Accession Number
2	cadherin 19 (NOTE: redefinition of symbol) {Incyte PD: 2902570}	2902570	AF047826
2	calmegin {Incyte PD: 2498216}	2498216	NM_004362
2	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein) {Incyte PD: 1806071}	1806071	X14831
2	CCAAT/enhancer binding protein (C/EBP), alpha {Incyte PD: 1831207}	1831207	AI193578
2	CD24 antigen (small cell lung carcinoma cluster 4 antigen) {Incyte PD: 1921393}	1921393	AA946784
2	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated) {Incyte PD: 951043}	951043	AA700158
2	ceruloplasmin (ferroxidase) {Incyte PD: 604991}	604991	AI684991
2	chondroitin sulfate proteoglycan 6 (bamacan) {Incyte PD: 937975}	937975	AA485586
2	claudin 1 {Incyte PD: 2257362}	2257362	AI925765
2	cleavage stimulation factor, 3' pre-RNA, subunit 3, 77kD {Incyte PD: 2718810}	2718810	U15782
2	coagulation factor C (Limulus polyphemus) homology (cochlin) {Incyte PD: 2844026}	2844026	AF006740
2	coagulation factor III (thromboplastin, tissue factor) {Incyte PD: 2061594}	2061594	AI085165
2	coagulation factor VIIIc, procoagulant component (hemophilia A) {Incyte PD: 1760838}	1760838	AA808551
2	collagen, type XIX, alpha 1 {Incyte PD: 4460789}	4460789	AB004628
2	copine III {Incyte PD: 3444952}	3444952	AB014536
2	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 10 (RNA helicase) {Incyte PD: 2346841}	2346841	NM_004398
2	deoxycytidine kinase {Incyte PD: 1832926}	1832926	AA883702
2	deoxycytidine kinase {Incyte PD: 1988022}	1988022	AI224043
2	DKFZP547E1010 protein {Incyte PD: 1730581}	1730581	AL050260
2	DKFZP564G1964 protein {Incyte PD: 2221768}	2221768	W47579
2	DKFZP564I1922 protein {Incyte PD: 1402105}	1402105	AA424901
2	DNA (cytosine-5-)-methyltransferase 1 {Incyte PD: 997561}	997561	NM_001379
2	dUTP pyrophosphatase {Incyte PD: 4861280}	4861280	AW276291
2	EGF-like-domain, multiple 5 {Incyte PD: 1743463}	1743463	AB011542
2	EphA5 {Incyte PD: 2149032}	2149032	L36644
2	ESTs {Incyte PD: 1215329}	1215329	AW003862
2	ESTs {Incyte PD: 1265073}	1265073	N63059
2	ESTs {Incyte PD: 1350974}	1350974	AA127809
2	ESTs {Incyte PD: 1379662}	1379662	AA587366
2	ESTs {Incyte PD: 1384677}	1384677	AA046458
2	ESTs {Incyte PD: 1403738}	1403738	AI937108
2	ESTs {Incyte PD: 1405820}	1405820	AI685256
2	ESTs {Incyte PD: 1413132}	1413132	AA058944
2	ESTs {Incyte PD: 1424411}	1424411	AI304430
2	ESTs {Incyte PD: 1429703}	1429703	AI480314
2	ESTs {Incyte PD: 1451756}	1451756	AA481964
2	ESTs {Incyte PD: 1507281}	1507281	W63702
2	ESTs {Incyte PD: 1554190}	1554190	AA934379
2	ESTs {Incyte PD: 1607729}	1607729	AA133894

Gene Expression Ratio	GeneName	CloneID	Accession Number
2	ESTs {Incyte PD: 1637856}	1637856	AA573432
2	ESTs {Incyte PD: 1638327}	1638327	AI336319
2	ESTs {Incyte PD: 1668733}	1668733	AI452519
2	ESTs {Incyte PD: 1677988}	1677988	AA403227
2	ESTs {Incyte PD: 1694382}	1694382	AA252126
2	ESTs {Incyte PD: 1720816}	1720816	AI375140
2	ESTs {Incyte PD: 1725256}	1725256	AI148143
2	ESTs {Incyte PD: 1739690}	1739690	T23939
2	ESTs {Incyte PD: 1808236}	1808236	AW084087
2	ESTs {Incyte PD: 1808653}	1808653	AW291290
2	ESTs {Incyte PD: 1809247}	1809247	AA927896
2	ESTs {Incyte PD: 1813721}	1813721	AA484891
2	ESTs {Incyte PD: 1849552}	1849552	AI017418
2	ESTs {Incyte PD: 1996163}	1996163	AA406528
2	ESTs {Incyte PD: 2019643}	2019643	AA629323
2	ESTs {Incyte PD: 2022039}	2022039	AA703046
2	ESTs {Incyte PD: 2089934}	2089934	AA041552
2	ESTs {Incyte PD: 2108138}	2108138	AI476248
2	ESTs {Incyte PD: 2110655}	2110655	AI640735
2	ESTs {Incyte PD: 2219955}	2219955	AI289575
2	ESTs {Incyte PD: 2253564}	2253564	AI739000
2	ESTs {Incyte PD: 2254021}	2254021	AW090060
2	ESTs {Incyte PD: 2290350}	2290350	AA233762
2	ESTs {Incyte PD: 2294294}	2294294	AI052511
2	ESTs {Incyte PD: 2306510}	2306510	AA742479
2	ESTs {Incyte PD: 2319475}	2319475	AI358791
2	ESTs {Incyte PD: 2348251}	2348251	AI051256
2	ESTs {Incyte PD: 2350560}	2350560	N29918
2	ESTs {Incyte PD: 2354433}	2354433	H98817
2	ESTs {Incyte PD: 2370225}	2370225	AA555042
2	ESTs {Incyte PD: 2445107}	2445107	AA250932
2	ESTs {Incyte PD: 2457149}	2457149	AA926994
2	ESTs {Incyte PD: 2472966}	2472966	AA988135
2	ESTs {Incyte PD: 2512249}	2512249	AI039268
2	ESTs {Incyte PD: 2555794}	2555794	AI039472
2	ESTs {Incyte PD: 2600615}	2600615	AI290443
2	ESTs {Incyte PD: 2632433}	2632433	AW295838
2	ESTs {Incyte PD: 2687892}	2687892	AA521146
2	ESTs {Incyte PD: 2729291}	2729291	AL079908
2	ESTs {Incyte PD: 2743036}	2743036	AA536187
2	ESTs {Incyte PD: 2756456}	2756456	AI057052
2	ESTs {Incyte PD: 2762703}	2762703	AI436281
2	ESTs {Incyte PD: 2763794}	2763794	AI689610
2	ESTs {Incyte PD: 2813837}	2813837	AI288937
2	ESTs {Incyte PD: 2816379}	2816379	AI684143
2	ESTs {Incyte PD: 2826117}	2826117	R71449
2	ESTs {Incyte PD: 2891379}	2891379	AI302221
2	ESTs {Incyte PD: 2902156}	2902156	AA085746

Gene Expression Ratio	GeneName	CloneID	Accession Number
2	ESTs {Incyte PD: 2951135}	2951135	AI823992
2	ESTs {Incyte PD: 2953987}	2953987	AI620789
2	ESTs {Incyte PD: 3143733}	3143733	AA856763
2	ESTs {Incyte PD: 3149777}	3149777	AW023860
2	ESTs {Incyte PD: 3204170}	3204170	AW006067
2	ESTs {Incyte PD: 3367716}	3367716	AI079331
2	ESTs {Incyte PD: 4000275}	4000275	AW292120
2	ESTs {Incyte PD: 4061967}	4061967	AI028661
2	ESTs {Incyte PD: 539690}	539690	AA829781
2	ESTs {Incyte PD: 546469}	546469	AA863329
2	ESTs {Incyte PD: 630051}	630051	AI363365
2	ESTs {Incyte PD: 630604}	630604	AA946757
2	ESTs {Incyte PD: 676007}	676007	N51843
2	ESTs {Incyte PD: 698061}	698061	AI680921
2	ESTs {Incyte PD: 720145}	720145	AW204623
2	ESTs {Incyte PD: 766891}	766891	T64957
2	ESTs {Incyte PD: 767388}	767388	AA780295
2	ESTs {Incyte PD: 777672}	777672	AA805635
2	ESTs {Incyte PD: 785456}	785456	AW015988
2	ESTs {Incyte PD: 866632}	866632	AI683660
2	ESTs {Incyte PD: 912951}	912951	AI421404
2	ESTs {Incyte PD: 944063}	944063	AI038703
2	ESTs, Highly similar to CGI-26 protein [H.sapiens] {Incyte PD: 2170202}	2170202	AI375177
2	ESTs, Highly similar to proteasome [H.sapiens] {Incyte PD: 1256558}	1256558	AI126562
2	ESTs, Moderately similar to !!!! ALU SUBFAMILY SP WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 2364392}	2364392	AA947028
2	ESTs, Moderately similar to CELL GROWTH REGULATING NUCLEOLAR PROTEIN [M.musculus] {Incyte PD: 2233551}	2233551	AI347185
2	ESTs, Moderately similar to unknown [R.norvegicus] {Incyte PD: 2252822}	2252822	AI452821
2	ESTs, Moderately similar to vacuolar protein sorting homolog r-vps33b [R.norvegicus] {Incyte PD: 2354491}	2354491	AI143138
2	ESTs, Weakly similar to !!!! ALU SUBFAMILY SQ WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 2243954}	2243954	AI675122
2	ESTs, Weakly similar to !!!! ALU SUBFAMILY SX WARNING ENTRY !!!! [H.sapiens] {Incyte PD: 1819267}	1819267	AI879646
2	ESTs, Weakly similar to 7-60 [H.sapiens] {Incyte PD: 1909443}	1909443	R53619
2	ESTs, Weakly similar to alternatively spliced product using exon 13A [H.sapiens] {Incyte PD: 2116280}	2116280	AA640244
2	ESTs, Weakly similar to cDNA EST yk486b9.3 comes from this gene [C.elegans] {Incyte PD: 958633}	958633	AA587284
2	ESTs, Weakly similar to CGI-104 protein [H.sapiens] {Incyte PD: 1366602}	1366602	AI416967
2	ESTs, Weakly similar to hypothetical protein, similar to [H.sapiens] {Incyte PD: 2171638}	2171638	AA169398
2	ESTs, Weakly similar to IgE receptor beta subunit [H.sapiens] {Incyte PD: 2349263}	2349263	AW025125
2	ESTs, Weakly similar to K02B2.3 gene product [C.elegans]	2667605	AW014209

Gene Expression Ratio	GeneName	CloneID	Accession Number
	{Incyte PD: 2667605}		
2	ESTs, Weakly similar to KIAA0309 [H.sapiens] {Incyte PD: 571157}	571157	AI274849
2	ESTs, Weakly similar to MaxiK channel beta 2 subunit [H.sapiens] {Incyte PD: 2728225}	2728225	AW297141
2	ESTs, Weakly similar to ORF YOR283w [S.cerevisiae] {Incyte PD: 566860}	566860	AI243362
2	ESTs, Weakly similar to rho-type GTPase-activating protein rhoGAPX-1 isoform 2 [H.sapiens] {Incyte PD: 625035}	625035	AA046743
2	ESTs, Weakly similar to similar to cyclin B like [C.elegans] {Incyte PD: 2756454}	2756454	AW341691
2	ESTs, Weakly similar to Sox-like transcriptional factor [H.sapiens] {Incyte PD: 2383568}	2383568	AW204140
2	ESTs, Weakly similar to STE20-like kinase 3 [H.sapiens] {Incyte PD: 2793922}	2793922	AA191319
2	ESTs, Weakly similar to tumor necrosis factor-alpha-induced protein B12 [H.sapiens] {Incyte PD: 1726853}	1726853	U55984
2	ESTs, Weakly similar to Unknown [H.sapiens] {Incyte PD: 1923470}	1923470	AI279177
2	ESTs, Weakly similar to YCR053w, len:514 [S.cerevisiae] {Incyte PD: 2547084}	2547084	AA868786
2	eukaryotic translation elongation factor 1 alpha 1 {Incyte PD: 447718}	447718	AA921845
2	fibrinogen-like 2 {Incyte PD: 1980117}	1980117	AI292063
2	frizzled (Drosophila) homolog 1 {Incyte PD: 1690530}	1690530	NM_003505
2	frizzled (Drosophila) homolog 7 {Incyte PD: 2736837}	2736837	AA927796
2	gamma-aminobutyric acid (GABA) A receptor, alpha 6 {Incyte PD: 5378974}	5378974	NM_000811
2	glutamyl aminopeptidase (aminopeptidase A) {Incyte PD: 3320987}	3320987	L12468
2	GRO3 oncogene {Incyte PD: 617159}	617159	X53800
2	H.sapiens polyA site DNA {Incyte PD: 1657705}	1657705	AA772217
2	hepatocyte growth factor (hepapoietin A; scatter factor) {Incyte PD: 4190790}	4190790	X16323
2	HIRIP5 protein {Incyte PD: 2502125}	2502125	AI818645
2	Homo sapiens clone 24421 mRNA sequence {Incyte PD: 1985427}	1985427	AF070641
2	Homo sapiens clone 24511 mRNA sequence {Incyte PD: 2317638}	2317638	AA744558
2	Homo sapiens clone 24538 mRNA sequence {Incyte PD: 729614}	729614	AW338844
2	Homo sapiens clone 24812 mRNA sequence {Incyte PD: 1969294}	1969294	AI983620
2	Homo sapiens DNA sequence from P1 p373c6 on chromosome 6p21.31-21.33. Contains zinc finger proteins, pseudogenes, ESTs and STS {Incyte PD: 2171634}	2171634	AI139333
2	Homo sapiens homeobox protein (HOX-1.3) gene, complete cds {Incyte PD: 3526675}	3526675	M26679
2	Homo sapiens mRNA from chromosome 5q21-22, clone:843Ex {Incyte PD: 2666301}	2666301	AB002449
2	Homo sapiens mRNA, chromosome 1 specific transcript KIAA0485 {Incyte PD: 3342469}	3342469	AB007954
2	Homo sapiens mRNA; cDNA DKFZp564H142 (from clone DKFZp564H142) {Incyte PD: 1416636}	1416636	F03819

Gene Expression Ratio	GeneName	CloneID	Accession Number
2	Homo sapiens mRNA; cDNA DKFZp564O1262 (from clone DKFZp564O1262) {Incyte PD: 2743751}	2743751	AA922441
2	Homo sapiens PAC clone DJ1159O04 from 7p21-p22 {Incyte PD: 814763}	814763	AI659972
2	Homo sapiens paired mesoderm homeo box 1 (PMX1), mRNA {Incyte PD: 3118038}	3118038	NM_006902
2	Human chromosome 5q13.1 clone 5G8 mRNA {Incyte PD: 1823641}	1823641	AI056697
2	Human clone 23760 mRNA, partial cds {Incyte PD: 1425925}	1425925	U79263
2	Human DNA sequence from clone 1052M9 on chromosome Xq25. Contains the SH2D1A gene for SH2 domain protein 1A, Duncan's disease (lymphoproliferative syndrome) (DSHP), part of a 60S Acidic Ribosomal protein 1 (RPLP1) LIKE gene and pa {Incyte PD: 2948040}	2948040	AL022718
2	Human DNA sequence from clone 273N12 on chromosome 6q16.1-16.3. Contains the gene for the N-Oct5a (N-Oct3, N-Oct5b) POU domain proteins and an unknown gene. Contains a putative CpG island, ESTs, STS, and GSSs {Incyte PD: 3074109}	3074109	AL022395
2	Human monocyte PABL (pseudoautosomal boundary-like sequence) mRNA, clone Mo1 {Incyte PD: 3629462}	3629462	AW296221
2	huntingtin-interacting protein 2 {Incyte PD: 1981594}	1981594	U58522
2	immunoglobulin (CD79A) binding protein 1 {Incyte PD: 1807922}	1807922	Y08915
2	Incyte EST {Incyte PD: 1443766}	1443766	AJ251961
2	Incyte EST {Incyte PD: 1738776}	1738776	AL035454
2	Incyte EST {Incyte PD: 1740889}	1740889	AL564563
2	Incyte EST {Incyte PD: 2060715}	2060715	NM_003069
2	Incyte EST {Incyte PD: 2189366}	2189366	AI207250
2	Incyte EST {Incyte PD: 2242287}	2242287	AW084810
2	Incyte EST {Incyte PD: 2243769}	2243769	
2	Incyte EST {Incyte PD: 3426983}	3426983	
2	Incyte EST {Incyte PD: 3606345}	3606345	
2	Incyte EST {Incyte PD: 610223}	610223	AI825820
2	Incyte EST {Incyte PD: 664237}	664237	AA026356
2	inositol 1,4,5-triphosphate receptor, type 2 {Incyte PD: 3002566}	3002566	D26350
2	KIAA0101 gene product {Incyte PD: 2458926}	2458926	D14657
2	KIAA0166 gene product {Incyte PD: 789903}	789903	D79988
2	KIAA0182 protein {Incyte PD: 1845755}	1845755	D80004
2	KIAA0335 gene product {Incyte PD: 2308348}	2308348	AB002333
2	KIAA0459 protein {Incyte PD: 913698}	913698	AI970454
2	KIAA0624 protein {Incyte PD: 2952693}	2952693	AI193238
2	KIAA0625 protein {Incyte PD: 2213276}	2213276	AI660878
2	KIAA0715 protein {Incyte PD: 2532486}	2532486	AB018258
2	KIAA0819 protein {Incyte PD: 1910948}	1910948	AI769376
2	KIAA0846 protein {Incyte PD: 1702714}	1702714	AB020653
2	KIAA0884 protein {Incyte PD: 1924277}	1924277	AB020691
2	KIAA0996 protein {Incyte PD: 2383661}	2383661	AA937190
2	KIAA1018 protein {Incyte PD: 2231573}	2231573	AI758219

Gene Expression Ratio	GeneName	CloneID	Accession Number
2	killer cell lectin-like receptor subfamily A, member 1 {Incyte PD: 3607409}	3607409	AF047445
2	leptin receptor {Incyte PD: 2843638}	2843638	AI453650
2	leukemia associated gene 2 {Incyte PD: 3035874}	3035874	Y15228
2	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A) {Incyte PD: 1998061}	1998061	AI952550
2	melanoma antigen, family A, 6 {Incyte PD: 1471808}	1471808	D32076
2	methyl-CpG binding domain protein 2 {Incyte PD: 517977}	517977	AI138289
2	methyltransferase-like 1 {Incyte PD: 1603584}	1603584	Y18643
2	microsomal triglyceride transfer protein (large polypeptide, 88kD) {Incyte PD: 1427623}	1427623	NM_000253
2	minichromosome maintenance deficient (S. cerevisiae) 2 (mitotin) {Incyte PD: 1723834}	1723834	AW264268
2	mitochondrial intermediate peptidase {Incyte PD: 1419396}	1419396	AA524277
2	mutS (E. coli) homolog 3 {Incyte PD: 2888235}	2888235	NM_002439
2	nescient helix loop helix 2 {Incyte PD: 551016}	551016	M96740
2	neuropeptide Y receptor Y2 {Incyte PD: 3630683}	3630683	U42766
2	NIMA (never in mitosis gene a)-related kinase 3 {Incyte PD: 1737403}	1737403	Z29067
2	nuclear body protein Sp140 {Incyte PD: 3992970}	3992970	U36500
2	Opa-interacting protein 5 {Incyte PD: 1909737}	1909737	AI419769
2	paired mesoderm homeo box 1 {Incyte PD: 3036857}	3036857	Z97200
2	paraoxonase 3 {Incyte PD: 1605789}	1605789	AI074616
2	peroxisomal acyl-CoA thioesterase {Incyte PD: 663478}	663478	AI628256
2	Phosphatidic acid phosphatase type 2a {Incyte PD: 1806438}	1806438	D29641
2	phosphoinositide-3-kinase, regulatory subunit, polypeptide 3 (p55, gamma) {Incyte PD: 2840772}	2840772	D88532
2	phosphotriesterase-related {Incyte PD: 3716611}	3716611	AI928486
2	polycystic kidney disease 2 (autosomal dominant) {Incyte PD: 1651294}	1651294	U50928
2	postmeiotic segregation increased 2-like 11 {Incyte PD: 1691161}	1691161	AW001329
2	postsynaptic protein CRIPT {Incyte PD: 2285977}	2285977	AI223792
2	potassium inwardly-rectifying channel, subfamily J, member 6 {Incyte PD: 2690251}	2690251	AA056665
2	proteasome (prosome, macropain) 26S subunit, non-ATPase, 5 {Incyte PD: 1811583}	1811583	D31889
2	proteasome (prosome, macropain) inhibitor subunit 1 (PI31) {Incyte PD: 1835394}	1835394	AI088066
2	protocadherin 8 {Incyte PD: 4090868}	4090868	AF061573
2	putative DNA binding protein {Incyte PD: 3031905}	3031905	AF073293
2	putative neuroblastoma protein {Incyte PD: 1732416}	1732416	D89016
2	putative protein {Incyte PD: 1995470}	1995470	AA046882
2	putative type II membrane protein {Incyte PD: 1372549}	1372549	AA448002
2	RAD51 (S. cerevisiae) homolog (E coli RecA homolog) {Incyte PD: 2258855}	2258855	D14134
2	rearranged L-myc fusion sequence {Incyte PD: 2835105}	2835105	U22377
2	reproduction 8 {Incyte PD: 2254530}	2254530	AI005235
2	Rho-associated, coiled-coil containing protein kinase 2 {Incyte PD: 1652328}	1652328	NM_004850
2	ribonuclease P (38kD) {Incyte PD: 1931267}	1931267	AI016477

Gene Expression Ratio	GeneName	CloneID	Accession Number
2	ribose 5-phosphate isomerase A (ribose 5-phosphate epimerase) {Incyte PD: 1863189}	1863189	L35035
2	S100 calcium-binding protein A7 (psoriasin 1) {Incyte PD: 1425169}	1425169	AW237898
2	sarcolipin {Incyte PD: 1867522}	1867522	U96094
2	serine/threonine kinase 24 (Ste20, yeast homolog) {Incyte PD: 2131876}	2131876	N34040
2	serine/threonine kinase 9 {Incyte PD: 2014951}	2014951	NM_003159
2	single-stranded DNA-binding protein {Incyte PD: 1371526}	1371526	AA649071
2	small nuclear ribonucleoprotein polypeptide G {Incyte PD: 2449837}	2449837	AL110340
2	small nuclear RNA activating complex, polypeptide 2, 45kD {Incyte PD: 1445203}	1445203	AI453282
2	spectrin, alpha, erythrocytic 1 (elliptocytosis 2) {Incyte PD: 2049446}	2049446	AA703344
2	SRY (sex-determining region Y)-box 10 {Incyte PD: 858309}	858309	AA976578
2	sterol carrier protein 2 {Incyte PD: 2507266}	2507266	AI659202
2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3 {Incyte PD: 2830336}	2830336	AI459249
2	target of myb1 (chicken) homolog-like 1 {Incyte PD: 1494830}	1494830	AI433695
2	TEK tyrosine kinase, endothelial (venous malformations, multiple cutaneous and mucosal) {Incyte PD: 2954469}	2954469	AL047086
2	thioredoxin-like {Incyte PD: 888182}	888182	W73191
2	thyroid hormone receptor interactor 8 {Incyte PD: 4003794}	4003794	Z39133
2	tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) {Incyte PD: 591358}	591358	AI952703
2	toll-like receptor 4 {Incyte PD: 2298442}	2298442	NM_003266
2	transcobalamin I (vitamin B12 binding protein, R binder family) {Incyte PD: 642823}	642823	NM_001062
2	transcription factor 9 (binds GC-rich sequences) {Incyte PD: 2779394}	2779394	M29204
2	transcription factor AP-2 gamma (activating enhancer-binding protein 2 gamma) {Incyte PD: 1921374}	1921374	AI638098
2	transforming growth factor, beta 2 {Incyte PD: 2360461}	2360461	Y00083
2	transforming growth factor, beta-induced, 68kD {Incyte PD: 2056395}	2056395	AC004503
2	translocase of inner mitochondrial membrane 8 (yeast) homolog A {Incyte PD: 698901}	698901	AL035422
2	trinucleotide repeat containing 12 {Incyte PD: 1967172}	1967172	AI143868
2	UDP glycosyltransferase 2 family, polypeptide B7 {Incyte PD: 1633719}	1633719	J05428
2	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 4 {Incyte PD: 1925068}	1925068	AI341667
2	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog {Incyte PD: 515453}	515453	AI740449
2	zinc finger protein 198 {Incyte PD: 1871741}	1871741	NM_003453

Functional clustering was used to assess differential gene expression in biochemical pathways (*e.g.*, cAMP and phosphoinositol pathways), protein families (*e.g.*

dopamine receptors); protein motifs (*e.g.*, zinc finger, leucine zipper, *etc.*) and transcripts previously identified as associated with cocaine abuse in human and animal models.

Reverse Northern Analysis was used as the secondary screen for 192 transcripts that were identified as differentially expressed (> 1.8 fold) in the high density microarray or as candidate genes that did not appear on the microarray. The resulting data are consistent with neuroadaptive response in intracellular signaling cascades associated with long-term cocaine-induced stimulation of dopamine receptors. Therefore, expression levels of a number of dopamine and signal transduction levels were determined by reverse Northern analysis to determine the pattern of gene expression and indicate whether the VTA in cocaine overdose victims is preferentially affected as a consequence of long-term cocaine use. mRNA from each subject for each group was hybridized separately to two reverse Northern blots each containing 96 candidate clones. PCR was performed to amplify the inserts of the clones of interest. Approximately 250 ng of PCR material from each clone was spotted on Hybond XL nylon membrane. Probes were generated as described for Example 1 and hybridized to reverse Northern blots. Blots were exposed to phospho screens and desitometric analysis was performed using ImageQuant software.

Secondary screening demonstrated significant alterations in the expression of multiple genes and of several function-related gene groups. Chronic cocaine exposure enhances DA neurotransmission, which may result in regulatory changes in postynaptic DA and D2 receptors. D1 and D2 receptors are coupled to Gαs and Gαi proteins, respectively, and function to stimulate and inhibit AC activity. The data demonstrate an increase in Gαs and Gαi mRNA in the VTA of cocaine overdose victims, as well as significant differences in γ2 and γ5 subunits.

RGS proteins regulate G-protein signaling by increasing the intrinsic GTPase activity of Gαi, Gαo, Gαt, and Gαq as well as Gsα. RGS1-4 and 10 showed greater than 2 fold differential expression in the VTA of cocaine overdose victims. Although the present invention is not bound by a particular mechanism, these data suggest that RGS proteins are located in dopamine pathways and may be involved in neuroadaptations in signal transduction mechanisms associated with chronic cocaine exposure.

Experimental Procedures

1. Target and Probe Preparation

cDNA from the clones are amplified, purified, and aliquoted into 96-well plates using a Quiagen BioRobot 3000. Universal primer pairs are used for PCR. Clones are amplified, and an aliquot is electrophoresed on an agarose gel for quality control, and any failed reaction (*e.g.* a reaction resulting in no or multiple bands) is repeated. cDNA is purified from the PCR reactions and an aliquot is used for fluorometric quantification. Samples are deleted in 50% DMS) to prevent evaporation during spotting. Plates containing the products are loaded onto a Cartesian Microarray and spotted at a concentration of 10 ng per spot. Each clone is spotted three times in different areas of the array. The arrayer uses a quill type spotter and is enclosed in a temperature and humidity controlled chamber. Pre-spotting 10 times increases the reproducibility of the amount of product spotted on arrays. Microarrays are dried at 37°C for one hour, and are then irradiated by ultraviolet to cross-link the DNA. Microarrays are washed with 70% ethanol, dried at 37°C and stored in dessicators at 4°C until needed.

To produce probes, approximately 400 ng of mRNA is incubated at 70°C for 20 minutes, then quick cooled in ice for 5 minutes. Oligo dT-24 (400 ng), 250 μM each of dATP, dGTP, dCTP, and Cy 3.5 or Cy 5.5 labeled dUTP (Amersham Pharmacia Biotech.), 10 mM DTT and reverse transcriptase buffer to a final concentration of 1X are added to the mRNA. The sample is incubated at 37°C for 30 minutes. On μl of SUPERSCRIPT ® brand RNase H- reverse transcriptase (Invitrogen) is added to the solution and incubated at 37°C for 90 minutes.

2. Hybridization

Hybridization of microarrays is performed using a Genomic Solutions GenTac Hybridization Staion. Both labeled probes are added to the hybridization chamber and are agitated genetly at 48°C for 24 hours. Following hybridization, a medium stringency was buffer (2X SSC/0.1% SDS) is applied to the slides with a buffer flow time of 45 seconds at 40°C for 6 minutes. A second was step with a buffer of higher stringency (0.5X SSC/0.1% SDS) is applied to the slides with a buffer flow time of 45 seconds at 40°C for 3 minutes. 2X SSC is used as the post wash buffer with a flow time of 40 seconds at 25° for 30 seconds each. Slides are dried at 25°C for 5 minutes before image analysis.

3. Image Analysis

Following hybridzation, microarrays are scanned using the GSI Lumonics ScanArray 5000. Initial image analysis is performed using ScanArray software, and are subsequently analyzed using GLEAMS software (NuTec Services). The levels of the Cy3 and Cy5 signals are determined, and the size and spacing of the dots on the microarray are defined and corrected for artifacts. The background is calculated for each spot using the immediate area surrounding the target spot. Controls are used to adjust channel intensity and to calculate the overall expression mean of each gene. Criteria for inclusion of spots for analysis include: 1) >4-fold signal intensity over background, 2) a symetric spot (not intensity) patter for each channel 3) lack of significant artifactual signal intensity (intensity recognition pattern algorithm in software and visual identification, and 4) hybridization present in 2 of the three spots per gene.

4. Reverse Northern Analysis

In order to produce blots for reverse northern analysis, clones are amplified and analyzed on agarose gels as described above. 250 ng of each amplified insert is spotted on HyBond XL ® net nylon transfer membrane (Amersham Pharmacia Biotech) using a 96 well dot blot apparatus (Schleicher and Schuell). DNA is crosslinked to the membrane by ultraviolet radiation. This amount of cDNA ensures that the cDNA is in vast excess to the respective aRNA, so that the target will not limit hybridization. Arrays will be hybridized for 48 hours at 44°C in a rotisserie hybridization oven with the following hybridization solution: 50% formamide, 5X SSC, 5X Denhard's solution, 0.1% SDS, 200 ng of sheared salmon sperm, and 1.0 mM sodim pyrophosphate. Following hybridization, slots blots are washed sequentially with 2X SSC/0.1% SDS, 0.5X SSC/0.1% SDS, and 0.1X SSC/0.1% SDS for 20 minutes each at 44°C. Labeled hybridized products are detected using phosphorimager cassettes, Image StormScanner and ImageQuant software (Molecular Dynamics).

5. Statistical Analysis

Expression levels from cocaine overdose victims and controls will be compared using univariate statistics for continuous variables and central tendency will be measured using the Wilcoxon signed rank sum test. Crude OR's and 95% confidence intervals will be estimated by the Mantel Haenszel method. 95% confidence intervals for all odds ratios are calculated with unconditional maximum likelihood. In order to determine the

significance of differential gene expression between control and cocaine exposure groups, experimental data will be analyzed by a regression analysis.

The specific signal (corrected for background) of probe bound to each clone is expressed as a ratio of the total hybridization intensity of the array, thereby minimizing variations due to differences in the specific activity of the probe and the absolute quantity of probe present. Differential expression greater than 1.8-fold is accepted as above background and relevant for further examination. Relative changes in individual mRNAs for the respective brain regions is analyzed using a two-way ANOVA (analysis of variance between groups) with Group (cocaine overdose, controls) and Region (VTA vs. l-SN; NAc vs. d-CP) as the fixed effects. The null hypothesis is rejected when $P<0.05$. Post hoc analyses are conducted as needed using Fisher least significance difference. Differentially expressed genes are grouped (clustered) into functional categories (*e.g.* signal transduction pathways, protein families such as receptors, RGS proteins, G-protein subunits, etc.) as well as to provide information on coordinate gene expression in a functional context. Differential gene expression is examined for relationships with indices of cocaine use (*e.g.* blood and brain concentrations; frequency and duration of use) by one-way ANOVA followed by post hoc analyses where appropriate. mRNA abundance serves as the dependent measure. Additional clinical data, including age, postmortem interval, sex, and race are examined for effects on gene expressing using ANOVA or correlation analysis, as appropriate. To confirm that the cocaine users and comparison subjects are well matched, age, sex, race, socioeconomic status, and postmortem interval will be compared by using two-tailed t tests. The parameters of the appropriate regression function (*i.e.*, linear or quasilinear) will be estimated and the correlation coefficient determined. Comparisons between means will be made using least significant difference with $P<0.05$. Similar analysis is performed by hemispheric laterality.

Example 3: Alterations in Ionotropic Glutamate Receptor Subunits during Binge Cocaine Self-Administration and Withdrawal in Rats

I. Introduction

Ionotropic glutamate receptors are classified as NMDA (NR1, NR2A-D, NR3), (±)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; GluR1-4), and kainate (GluR5-7, KA1-2) receptor subunits based on their pharmacological characteristics and sequence information (Hollmann and Heinemann, S. (1994) *Annu. Rev. Neurosci.* 17, 31-108. Borgesmand Dingledine (2002) Molecular pharmacology and physiology of glutamate receptors, in *Glutamate and addiction*, Herman *et al*, eds, pp 3-22. Humana Press, Totawa, NJ). Whereas AMPA and kainate subunits contribute to fast neurotransmission, all three ionotropic subtypes are thought to play roles in long term potentiation. Since subunit composition determines the functional properties of ionotropic glutamate receptors, alterations in ionotropic glutamate receptors in the VTA may indicate alterations in the excitability of dopamine transmission underlying long term biochemical and behavioral effects of cocaine which, in turn, may affect subsequent drug intake.

The effect of cocaine self-administration on alterations in the abundance of glutamate receptor subunits has not been described in the art. The present study was undertaken to evaluate changes in the abundance of iGluR subunit expression in mesocorticolimbic structures involved in cocaine reinforcement (VTA, NAc, PFC) following binge cocaine self-administration and withdrawal from cocaine. Similar changes were evaluated in the nigrostriatal pathway (SN and dorsal striatum) as a measure of regional and pathway specificity.

II. Materials and Methods

A. Subjects

Male Sprague-Dawley rats (60-90 days; 225-275g; Charles River, Wilmington, MA) were housed individually in operant conditioning cages in a temperature-controlled vivarium on a 12 hr reversed light-dark cycle (lights on: 8:00 p.m.) with food and water available *ad libitum* throughout the experiment.

B. Intravenous catheterization

Rats were anesthetized with halothane and implanted with chronic indwelling venous catheters, as described previously (Hemby *et al.* (1999) *J. Pharmacol. Exp. Ther.* 288:274-80; Hemby *et al.* (1995) *J. Pharmacol. Exp. Ther.* 273:591-98; Hemby (1997b) *Psychopharmacology* (Berl). 133:7-16). Catheters were inserted into the right jugular vein, terminating just outside the right atrium and anchored to muscle near the point of entry into the vein. The distal end of the catheter was guided subcutaneously to exit above the scapulae through a Teflon shoulder harness. The harness provided a point of attachment for a spring leash connected to a single channel swivel at the opposing end. The catheter was threaded through the leash for attachment to the swivel where the fixed end of the swivel was connected to a syringe by polyethylene tubing. Infusions were administered by a motor driven syringe pump controlled by a computer. Infusions of methohexital (100 μl; 10 mg/kg; i.v.) were administered to assess catheter patency, as needed. Health of the rats was monitored thrice daily by the experimenter and biweekly by institutional veterinarians according to the guidelines issued by the Emory University Institutional Animal Care and Use Committee and the National Institute of Health.

C. Self-administration Procedures

Subjects were housed in standard operant conditioning chambers (24.5x23.5x21cm) containing a retractable lever and a stimulus light mounted directly above the lever. The chambers were enclosed in sound-attenuating boxes containing an exhaust fan, a house light, a tone source, and a water bottle. A motor driven syringe pump was located on the side of this external chamber. Extraneous noise was masked by the exhaust fan. Immediately following surgery, rats were placed in their respective chambers where they received infusions of heparinized 0.9% bacteriostatic saline (1.7 U/ml; 200 μl/30min) for 48 hr. On the following day, the self-administration procedure was initiated.

Rats were randomly divided into two groups: (BINGE access and WITHDRAWAL) and allowed to self-administer cocaine (0.5 mg/infusion; 200 μl/infusion; 6.2 sec/infusion) during an 8 hr. self-administration sessions (dark phase of the light cycle) under a fixed ratio-5 (FR5); time out 20 seconds schedule of reinforcement. Upon completion of the response requirement, a cocaine infusion was

delivered and a 20-sec time-out was in effect. Responding was initially maintained under an FR1 that was gradually increased to FR5. During the time-out, the lever light was extinguished, the house light illuminated, and a tone was generated. The end of the time-out was signaled by illumination of the lever light and the house light and tone were extinguished. During the time-out, lever responses were recorded but had no scheduled consequence. IBM compatible computers were used for session programming and data collection. Once attaining the terminal ratio of FR5, rats were given limited access (8hrs/day, 7days/week) for 14 consecutive days. On the 15th day, the self-administration session was changed to multiple 3 hr access components separated by one hr time outs (TO) as depicted below:

Dark Cycle (8:31 a.m. to 8:30 p.m.)						Light Cycle (8:31 p.m. to 8:30 a.m.)					
3 hr access self-admin	TO	3 hr access self-admin	TO	3 hr access self-admin	TO	3 hr access self-admin	TO	3 hr access self-admin	TO	3 hr access self-admin	TO

Twenty-four sessions were looped such that the program began each day at the beginning of the dark cycle. After completion of the self-administration session on the 20th day, rats in the BINGE access group were sacrificed. On days 21-34, rats in the WITHDRAWAL group remained in the self-administration chambers but did not have access to cocaine or related stimuli and were sacrificed on day 35.

D. Tissue preparation and Western blot analysis

For sacrifice, rats were anesthetized with halothane and intracardially perfused with phosphate buffered saline (pH=7.2). Brains were removed and sectioned on ice in the coronal plane using a brain matrix. Areas of interest were dissected immediately on ice-cooled aluminum plates from 1-mm slices (approximately +3.2 to +2.2, PFC; +1.7 to 0.7, NAc; 0.48 to -0.4, striatum; -5.2 to -6.2, SN/VTA; all measures relative to bregma (Paxinos and Watson (1998) The rat brain in stereotaxic coordinates, 4th edition, Academic Press); and immediately frozen at -80°C in Eppendorf tubes.

The sample size for the Binge, Withdrawal and Control groups for the NAc, PFC and striatum was n=8 per region. Due to the size of the VTA and SN, two samples were pooled such that n=4 for the Withdrawal, Binge and control groups for these regions. Tissue samples were homogenized in 10 mM HEPES, 10 mM NaCl, 1 mM KH_2PO_4, 5 mM $NaHCO_3$, 1mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM EDTA and the following protease inhibitors (PI): 1mM phenylmethylsulfonylfluoride, 10 mM benzamidine, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 μg/ml pepstatin and centrifuged using a Beckman Coulter SW55Ti swinging bucket rotor at 5333 *g* for 5 min. Supernatant (cytosol and crude membrane) was removed and centrifuged at 59,255 *g* for 30 min at 4°C and the pure cytosolic supernatant was removed and stored at -80°C. The pellet containing the crude plasma membrane was re-suspended in 20 mM Tris HCl, 1 mM EDTA (pH=8.0) and 300 mM sucrose with PIs and centrifuged at 5333 *g* for 5 min. This procedure was repeated twice and the pellet was re-suspended in phosphate buffered saline and stored at -80°C (crude plasma membrane fraction). The pellet from the intial centrifugation was re-suspended in 10 mM Tris (pH=7.5), 300 mM sucrose, 1 mM EDTA (pH=8.0), 0.1% NP40 and PIs and centrifuged at 2370 *g* for 5 min at 4°C. The supernatant was discarded and the pellet was re-suspended in the buffer and washed three times before re-suspension in the PI buffer and storage of samples at -80°C (nuclear fraction), as described previously in Tang *et al.* (2003) *J. Neurochem.* 85:911-24.

Protein concentrations were calculated using the bicinochoninic acid protein assay kit (Pierce, Rockford, IL) and diluted in Laemmli sample buffer to achieve the equivalent final protein concentrations. Five micrograms of protein were loaded into 10% sodium dodecyl sulfate-polyacrylamide gel, electrophoresed and transferred to nitrocellulose by electroblotting (30V, overnight at 4°C) in 1X transfer buffer (Bio-Rad, Richmond, CA). Nitrocellulose membranes were blocked in 0.5% w/v nonfat dry milk and 0.1% v/v Tween 20 in phosphate-buffered saline (pH 7.4, 0.12 M) for 1 hr at room temperature prior to being incubated with primary antibodies in blocking buffer (Bio-Rad) overnight at 4°C followed by secondary antibody for one hr at room temperature. Protein bands were visualized on a Kodak XAR-5 film with enhanced chemiluminescence (ECL plus, Amersham Pharmacia Biotech). Primary antibodies were as follows: mouse monoclonal antibodies directed against NMDAR1 (Chemicon International, Temecula, CA) and rabbit polyclonal antibodies directed against

NMDAR2A, NMDAR2B, NMDAR3A, NMDAR3B, GluR1, GluR2/3, GluR4, GluR5, GluR6/7, and KA2 (Upstate Biotechnology Cell Signaling Systems). Equal protein loading was confirmed by stripping the blots and re-probing them with a monoclonal β-tubulin antibody (Upstate Biotechnology Cell Signaling Systems, Waltham, MA; 1:5000 v/v) followed by incubation with secondary antibody and visualization as described above. No significant differences were detected in ß-tubulin abundance between the groups for any of the blots indicating that any differences in ionotropic glutamate receptor abundance between the groups was not due in unequal loading of protein in the gels. Protein abundances were calculated by optical densitometry with a Scan Jet 2200C and imported into NIH Image 1.61 software for analysis. Film background was subtracted from the optical density values to give the optical density value for each subject. All assays were conducted under conditions in which densitometric signal intensity was linear with protein concentration as determined by preliminary experiments. Data were expressed as percent of control levels (mean ± S.E.M.).

E. Data Analysis

Self-administration data were analyzed by two-way ANOVA with repeated measures (Time) with number of infusions as the dependent measure. Data for each iGluR subunit/region were normalized based on control levels and were analyzed using one way ANOVA. Null hypotheses were rejected when $P<0.05$.

III. Results

A. Behavioral data.

Cocaine engendered and maintained rates of self-administration. There was no statistically significant difference in the number of infusions, and likewise cocaine intake, between the two groups over the course of the experiment [$F(1, 377)=0.401$, $P=0.544$]. T he total number of infusions was 895.8 (± 101.7) for the BINGE group and 981.3 (± 232.9) for the WITHDRAWAL group. During the 15 days of limited access, rats in the BINGE group averaged 551.3 (± 60.9) infusions/275.7 (± 30.5) mg of cocaine; whereas the WITHDRAWAL group averaged 621.7 (± 42.7) infusions/310.9 (± 21.4) mg of cocaine. Similarly, during the six days of unlimited access, the BINGE group self-administered approximately 344.4 (± 48.3)

infusions/172.2 (± 24.2) mg of cocaine and the WITHDRAWAL group self-administered 359.5 (± 48.0) infusions/179.8 (± 24.0) mg of cocaine.

B. Regional comparison of iGluR subunit protein levels in controls.

Analysis of the relative abundance of iGluR subunits across various brain regions was performed. Western blots of the iGluR subunits in each of the brain regions studied revealed single bands at the appropriate molecular weight. For the NMDA subunits, NMDAR1 was most abundant in the hippocampus, followed by the PFC, the NAc and striatum (HIPP>PFC>NAc, striatum>> VTA, SN). There were no apparent differences in abundances of NMDAR2A, 2B, or 3A among the hippocampus, PFC, NAc and striatum while these subunits were in low abundance in the VTA and SN. Interestingly, the NMDAR3B subunit appeared to be most abundant in the PFC and striatum followed by the hippocampus and NAc. Due to the paucity of protein from the VTA and SN, NR3A and 3B levels were not assessed. The abundances of GluR1, GluR2/3 and GluR4 were greater in the hippocampus and PFC than the NAc and striatum (HIPP, PFC>NAc, striatum>VTA, SN). GluR5 protein levels appeared to be equally abundant in all regions tested. For the kainate receptor subunits, GluR6/7 was most abundant in the NAc, striatum and PFC followed by the hippocampus, then the SN and VTA. In contrast, KA2 was most abundant in the NAc, moderately abundant in the striatum, PFC and hippocampus and least abundant in the VTA and SN.

C. Effects of cocaine self-administration history on iGluR subunit levels

NR1: Analysis of variance revealed a significant effect of cocaine history on protein levels in the SN [$F(2,16)=9.785$; $P=0.002$] and striatum [$F(2,22)=11.217$; $P<0.001$]. No significant differences were observed in the VTA, NAc or PFC. *Post hoc* analyses revealed increased levels in the SN following binge access which returned to control levels following withdrawal, whereas levels in the striatum following binge access and withdrawal were both significantly greater than control levels.

NR2B: There was a significant effect of cocaine history on NR2B levels in the striatum [F(2,22)=12.996; P<0.001] and PFC [F(2,18)=11.881; P<0.001]. Similar to NR1, NR2B levels in the striatum were increased following binge access and withdrawal; both at levels significantly greater than control levels. In the PFC, levels were significantly greater following withdrawal than either control or binge access.

NR3A: Analysis of variance revealed a significant effect of cocaine history on protein levels in the striatum [F(2,22)=12.169; P<0.001] and PFC [F(3,26)=3.928; P=0.021]. Due to the relatively small amounts of available protein in the VTA and SN, NR3A levels were not assessed in these regions. In the striatum, binge access produced significantly greater levels compared to control and withdrawal. In the PFC, protein levels were significantly reduced following withdrawal below levels observed in controls and binge access.

NR3B: Protein levels were significantly altered by cocaine in the striatum [F(2,23)=101.821; P<0.001]. No changes were observed in the other brain regions. As noted for NR3A, levels were not assessed in the VTA or SN. Levels following binge access were significantly greater than withdrawal which, in turn, were greater than control levels.

GluR1: Analysis of variance revealed a significant effect of cocaine history on protein levels in the VTA [F(2,16)=5.924; P=0.014], SN [F(2,19)=8.890; P=0.002], striatum [F(2,22)=42.004; P<0.001] and PFC [F(2,20)=4.431; P=0.027], while there was no significant difference in the NAc. In the VTA, GluR1 levels were significantly greater during withdrawal compared with binge access. In contrast, GluR1 levels in the SN were increased during binge access but returned to control levels during withdrawal. In the striatum, levels were significantly elevated during withdrawal only (Figure 4A).

GluR2/3: Protein levels were significantly altered in the VTA [F(2,11)=5.494; P=0.028], NAc [F(2,22)=5.105; P=0.016], striatum [F(2,22)=38.918; P<0.001] and prefrontal cortex [F(2,20)=6.953; P=0.006], although no significant differences were observed in the SN. In the VTA, NAc, and PFC, withdrawal

produced significantly greater levels than binge access. In the striatum and PFC, GluR2/3 levels following withdrawal were significantly more abundant than control levels.

GluR4: ANOVA revealed significant effects of cocaine on GluR4 protein levels in the striatum [F(2,33)=23.629; P=0.007] and PFC [F(2,19)=8.211; P=0.003]. There was no significant difference in the VTA, SN or NAc. Similar patterns of changes were observed in the striatum and PFC with levels following withdrawal significantly increased above both control and binge levels.

GluR5: There was a significant effect of cocaine history on GluR5 levels in the VTA [F(2,11)=5.583; P=0.027], SN [F(2,16)=4.032; P=0.041] and striatum [F(2,16)=10.706; P=0.002]. No significant differences between the groups were observed in the NAc or PFC. In the VTA, levels were significantly decreased during binge access compared with control levels. In contrast, GluR5 levels in the SN increased during binge cocaine access and returned to control levels during withdrawal. In the PFC, levels were significantly increased during withdrawal above both control and binge levels.

GluR6/7: ANOVA revealed significant effects of cocaine on GluR6/7 protein levels in the VTA [F(2,11)= 20.731; P<0.001], SN [F(2,20)=4.232; P=0.031], and striatum [F(2,22)=23.934; P<0.001]. No significant differences were observed in the PFC or NAc, although there was a trend towards significance in the NAc [F(2,22)=3.339; P=0.056]. In the VTA, protein levels were significantly decreased during binge access but returned to control levels following two weeks of withdrawal. In contrast, SN GluR6/7 levels increased following binge cocaine access and returned to control levels during withdrawal. In the striatum, GluR6/7 protein levels were increased above control and binge access levels following two weeks of withdrawal (Figure 6B).

KA2: ANOVA revealed significant effects of cocaine on protein levels in the SN [F(2,20)=11.357; P<0.001] and striatum [F(2,22)=42.004; P<0.001]. There were no significant differences in KA2 levels between the groups in the VTA, NAc or PFC.

Levels were significantly increased in the SN during binge access and returned to control levels following withdrawal. In contrast, KA2 protein levels were significantly increased during withdrawal but were not different from control levels following binge access (Figure 6C).

5

Table 6: Human homologs of NMDA receptors showing significant regulation during Binge Cocaine-self administration and Wtihdrawal in Rats	
Gene Name	*Accession Number*
GRIN (encoding NMDAR1)	R88267
GRIN2B (encoding NMDAR2B)	NM_000834
GRIN2C (encoding NMDAR3)	L76224
NMDAR2D	U77783
mGLUR6	U82083

IV. Discussion

10 In the present study, Western blot analysis was used to examine the expression of iGluR protein subunits following binge cocaine self-administration and two weeks of withdrawal in brain the mesocorticolimbic and nigrostriatal dopamine pathways. The study demonstrated that binge cocaine self-administration and withdrawal induce changes in protein levels of iGluR subunits in a region specific manner and were 15 dependent upon the history of cocaine exposure. The present results provide the first composite assessment of iGluR subunit protein alterations in mesocorticolimbic brain regions associated with cocaine reinforcement (VTA, NAc, and PFC) compared with regions in the nigrostriatal pathway (SN and striatum).

20 A. NMDA receptor subunits

In the present study, no significant differences were observed in the VTA, NAc or PFC NR1 levels or following binge cocaine self-administration. However, NR1 protein levels were increased following six days of binge cocaine access in the SN compared to control and withdrawal levels and in the striatum following binge 25 and withdrawal compared to control levels. Interestingly, increased levels returned to baseline following 2 weeks of withdrawal in the SN whereas levels remained elevated following withdrawal in the striatum.

In the present study, NR2B and NR3B levels were increased in the striatum following binge cocaine self-administration and remained elevated above control levels following two weeks of withdrawal suggesting these changes may represent long-term alterations in NMDA receptor function following cocaine exposure. In contrast, NR3A levels in the striatum were increased following binge cocaine access but returned to control levels following two weeks of withdrawal. In the PFC, withdrawal from cocaine increased NR2B above control and binge access levels whereas withdrawal levels of NR3A were decreased compared to control and binge access levels. The presence of NR3A subunits, which must co-express with NR1 for membrane expression, leads to decreased Ca^{2+} permeability through the NMDA receptor complex. Since increased Ca^{2+} permeability is necessary for long-term synaptic changes, the present data suggest an increased opportunity for LTP to occur in mPFC neurons. From a functional perspective, increased NR2B levels indicate a slow deactivation time (~400 msec) for the receptor complex, which when paired with decreased expression of NR3A may yield an increase in Ca^{2+} permeability and hyperexcitability of prefrontal function. NR1 subunits are required for the normal function of the NMDA ionophore. The subunit is phosphorylated by PKA, PKC and possibly by CamKII which cause increased Ca^{2+} influx through the activated receptor leading to the slow onset of the EPSP. NR1 subunits may lead to enhanced Ca^{2+} influx in dopamine neurons resulting in hyperexcitability of these cells. With no apparent change in NR1 protein levels, NR3A alterations may provide an alternative means to increase intracellular Ca^{2+} levels without altering the number of receptors.

B. AMPA receptor subunits

GluR1 and GluR2/3 levels in the VTA were significantly increased during withdrawal compared with binge access. Similarly in the NAc, GluR2/3 levels were increased following withdrawal from cocaine compared with binge access, an effect also observed in human cocaine overdose victims. Significant upregulation of GluR2/3 and GluR4 in the PFC were observed following withdrawal compared with control and binge access levels.

In the nigrostriatal pathway, GluR1 levels in the SN were increased during binge access compared to control and withdrawal levels whereas AMPA subunit levels in the striatum were increased during two weeks of withdrawal from cocaine

compared to control and binge access levels. In addition, binge GluR2 levels in the striatum were significantly lower than control and withdrawal levels. Previous studies using experimenter-administered cocaine, did not observe significant alterations in AMPA subunits in the nigrostriatal pathway following chronic cocaine or withdrawal (Fitzgerald *et al.* (1996) *J. Neurosci.* 16,:274-82; Churchill *et al.* (1999). *J. Neurochem.* 72:2397-403), again suggesting the potential relative importance of contingent drug administration.

C. Kainate receptor subunits

GluR6 and KA2 exhibited similar moderate to strong immunoreactivity in the NAc, striatum and PFC and light immunoreactivity in the SN and VTA. GluR5 levels were moderate to strong in the PFC and were similarly expressed in the NAc, striatum, VTA and SN. Interestingly, GluR5 levels were expressed at considerably higher levels in the rat compared with human VTA. Unlike NMDA and AMPA receptor subunits, the physiological function of kainate receptors remains unclear; however, they appear to be important for controlling Ca^{2+} influx through the kainate ionophore in different pathological states, such as cocaine addiction (Paschen and Djuricic 1994).

In the present study, GluR5, GluR6/7 and KA2 kainate receptor subunit levels were regulated in a region specific manner. In the VTA, GluR5 and GluR6/7 immunoreactivities were decreased during binge access, whereas binge access increased GluR5, GluR6/7 and KA2 in the SN compared with withdrawal levels. Conversely, the kainate receptor subunits in the striatum were significantly upregulated during withdrawal compared with control and binge access levels. Presently, there is a paucity of information on the regulation and function of kainate receptor subunits and even less about the role of these subunits in cocaine abuse and addiction.

V. Summary

The present study demonstrated the regional and subunit-specific changes in iGluR protein expression following binge cocaine self-administration and withdrawal. Theseresults provide a significant addition to the knowledge of altered glutamatergic function induced by chronic cocaine self-administration and withdrawal. The study is

unique in that it is the first study to assess alterations of multiple iGluR subtypes in various brain regions following binge cocaine self-administration and withdrawal. Such changes may be related to behaviors associated with withdrawal such as decreased locomotion, increased anxiety and behavioral sensitization and other enduring effects and may be an important mechanism by which cocaine exerts long-term effects on the mesolimbic dopamine system.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.